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BULLETIN OF
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OF MEDICINE

DECEMBER 1940

EXPERIMENTAL BASIS OF CHEMOTHERAPY IN THE TREATMENT OF BACTERIAL INFECTIONS*

The Ludwig Kast Lecture

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THE use of sulfonamide drugs in the treatment of bacterial infections is based upon studies involving the effect of the drugs upon experimental infections in animals, the effect of the drugs upon various bacteria *in vitro*, and the effect of the drugs upon the host organism. This last includes a determination of the acute and chronic toxicity of a drug for several species of animals, its action on individual organs and tissues, its absorption, excretion and distribution, and the changes undergone by the drug in passing through the host. In all of these studies, it has been of the greatest value to have available accurate and simple methods for determining drug concentrations in blood, urine, body fluids and tissues.[1, 2, 3, 4, 5] These methods, I think, have been responsible more than anything else for placing the schedule of dosage of bacterial chemotherapeutic agents on a rational basis.

Nearly all research directed toward the chemotherapy of experi-

* Read October 14, 1940 in the Graduate Fortnight of The New York Academy of Medicine.

mental bacterial infections has utilized the mouse as the experimental animal. In the original observations of Domagk[6] on Prontosil, the discovery of the antibacterial activity of sulfanilamide,[7] the observations of Colebrook and Kenny,[8] Buttle, Gray and Stephenson[9] and Long and Bliss[10] on experimental streptococcus infections, and the study of the activity of sulfapyridine in pneumococcus infection,[11] all utilized the mouse to furnish the basis for the clinical trial of these three drugs. In the above cases, the transfer of results from the lowly mouse to man has been justified by subsequent clinical experience, but this will probably not always be so.

Relatively simple methods of treating infected mice suffice for detecting the antibacterial activity of a drug against the particular organism used, but a more elaborate procedure is necessary to evaluate such activity quantitatively. In the early studies of the influence of sulfanilamide on pneumococcus infections in mice, there is agreement of opinion that the drug possessed some activity; however, most observers reported that although death could be delayed in treated mice, recovery from the infection did not occur. The impression gained from these studies was that sulfanilamide had slight therapeutic value against the pneumococcus and apparently this prevented its thorough trial in pneumonia. Recently, in a careful study of mouse infection with forty-seven strains of the thirty types of pneumococci, Schmidt and Hilles[12] found that the therapeutic effectiveness of sulfanilamide was considerable; in some cases 100 per cent survivals resulted. They administered the drug every 6 hours, day and night, maintaining a blood concentration of 8 to 12 mgm. per cent, and showed that the infrequent administration of the drug was responsible for the failure of earlier observers to obtain beneficial effects. We have little data on the clinical value of sulfanilamide in pneumonia because sulfapyridine has already established itself. However, in the one careful study of its use, very good results are reported.[13]

In order to use *in vivo* studies of the sulfonamide derivatives against various bacteria in the mouse to point the way to the selection of the most effective drugs for clinical trial, it is necessary to have an accurate quantitative method for the evaluation of the effectiveness of various compounds. Data on the effectiveness of drugs in infected mice have usually been obtained by administration of doses of the drugs according to schedules which have differed with each investi-

gator. Marked variation in blood concentrations of drug must have resulted from the different dosage schedules employed as well as from differences in the absorption, excretion and distribution of the various drugs. Another difficulty has been the lack of a suitable therapeutic response for an end-point. Recently, we have developed in our laboratory a method for the quantitative evaluation of chemotherapeutic agents in bacterial infections in mice which appears to give a more or less absolute comparison of two drugs independent of many variables within the experiment.[14, 15, 16] The method need not be described as it is given in detail in the communications to which reference is made. The quantitative data for several drugs in a streptococcus and a pneumococcus infection in mice appear to indicate some specificity on the part of these drugs for different bacterial infections.

Numerous *in vitro* studies of the effect of various sulfonamide drugs have been reported against bacteria. These have had two purposes, an elucidation of the mode of action, and an appraisal of the drug for clinical use. However, the concentration of a drug necessary to produce a bacteriostatic or bactericidal action *in vitro* may have absolutely no apparent relation to that necessary for its *in vivo* activity. Concentrations of sulfanilamide of 10 to 20 mgm. per cent may result, in experiments *in vitro* on streptococci, in no effect, in bacteriostasis or in bactericidal effects, while much lower blood concentrations are effective in the treatment of streptococcus peritonitis in mice. We know now that the strain of streptococcus, the size of the initial inoculum, the composition of the medium (particularly the amount of antisulfanilamide factor present), and the temperature at which the test is performed have a marked effect on the result obtained.[17, 18] The question arises as to whether the *comparative* activity of different drugs *in vitro* may not give information concerning their relative activity *in vivo* on the same organism. Unfortunately, little quantitative information is available to answer this query. Sulfapyridine is about two or three times as active as sulfanilamide *in vitro*, but the two drugs show equal activity *in vivo* in the mouse against the same strain of streptococcus. Diaminodiphenylsulfone and sulfathiazole show many times the activity of sulfanilamide *in vitro*, but the former is only three times as active and the latter is no more active than sulfanilamide *in vivo* in the mouse.[15] It is probable that high bacteriostatic or bactericidal activity *in vitro* is only one of the prerequisites for high *in vivo* activity. For systemic infections, it appears

that *in vivo* studies in the mouse are more valuable than *in vitro* studies in pointing the way to the selection of the most effective drugs for clinical trial.

In the treatment of urinary tract infections and infections confined to the intestine, as well as in the local use of these drugs in infected wounds, the comparative *in vitro* activity of different sulfonamide drugs may be valuable as an aid for selecting drugs for clinical trial, as body defences may well play a minor role under these conditions. The greater *in vitro* activity of sulfapyridine[19] and sulfathiazole[20, 21] on bacteria found in urinary infections suggests that, other things being equal, these two drugs would be superior to sulfanilamide in treating urinary infections. Recently, in the course of a study of sulfanilylguanidine as a possible intestinal chemotherapeutic agent, we[22] found that this drug caused a marked reduction of the coliform organisms in the stools of mice fed the drug in their diet. It seems probable that pathogenic organisms in the intestinal tract which are more susceptible to sulfanlylguanidine *in vitro* than E. coli will likewise be more susceptible in the intestinal tract. In the case of such diseases as subacute bacterial endocarditis caused by Streptococcus viridans, with which one cannot produce satisfactory infections in experimental animals, one must rely upon the unsatisfactory *in vitro* studies of the activity of drugs.

It is obvious, however, that other factors besides comparative chemotherapeutic activity in mice and bacteriostatic or bactericidal activity *in vitro* must of necessity govern the selection of the most suitable drug for clinical trial in man. Such factors are contained in a study of the drug on the host. An assessment of the probable toxicity of a drug in the human being is of prime importance. In the first communication on the pharmacology of sulfapyridine,[23] this drug was stated to be about one-fourth as toxic as sulfanilamide. However, the poor absorption of sulfapyridine from the gastrointestinal tract prevents a determination of its true toxicity when the drug is given *per os*. By the use of a soluble sodium salt of sulfapyridine it has been shown that on the basis of blood concentration, this drug is more toxic acutely for mice, rabbits, and dogs than is sulfanilamide.[24] No information is available as to the chronic toxicity of sulfapyridine based on a maintained and known blood concentration of the drug. However, studies have been made of the effect of the repeated administration of one dose per day by

mouth:[25, 26] these have shown no toxicity except that resulting from the deposition of acetylsulfapyridine in the form of concretions and stones in the urinary tract of rats,[27] rabbits and monkeys. It is interesting to note that clinically sulfapyridine appears to be considerably more toxic than sulfanilamide.[28]

Sulfathiazole is less toxic acutely than sulfapyridine for mice[29, 30] and dogs; its chronic toxicity for mice[29] and rabbits appears to be greater and for rats and monkeys[29] less than that of sulfapyridine. However, the study of chronic toxicity of these drugs is complicated by the formation of acetyl-derivatives which may cause mechanical damage to tissues.

The above examples of determination of toxicity on animals illustrate how difficult it is to transfer such data to the human subject. However, if careful studies of chronic toxicity be made in several species of animals with known and maintained blood concentrations of free and conjugated drug, with careful pathological examination of tissues at the end of the experiment, and especially if attempts are made to determine the kind of toxicity which does not kill the animals—effects on blood, cerebral cortex, vomiting center, spinal cord, peripheral nerves, liver, kidneys, etc.—it would appear possible to make fairly accurate prediction for the human subject. In the above discussion of the toxicity of these drugs, evidence of toxicity has been usually based upon death of the animals, symptoms of toxicity observable with the naked eye, and upon pathological changes in the tissues. However, evidence is available that one drug may be more toxic than another to one system of the body, while the reverse may be true with another system. For example, sulfapyridine is about twice as toxic acutely as sulfanilamide on the basis of the death of mice,[24] yet sulfapyridine is only one-half as toxic to the hematopoietic system as sulfanilamide.[31] The use of the so-called chemotherapeutic index, which expresses the ratio between the dosage producing death and the dosage producing a therapeutic response, is of little value, and can be extremely misleading. Of course, it is at present impossible to predict hypersensitiveness and idiosyncrasy that may result from the drug in man. Determinations of the toxicity on animals should be comparative; sulfanilamide or sulfapyridine, the human toxicity of which is fairly well known, can be used as standards.

It is necessary to have information concerning the absorption, excretion, and distribution of these drugs before they can be used most effi-

ciently in the treatment of disease. It now seems proven that the maintenance of a certain concentration of drug in blood and tissues for several days is most efficient for treating infections. To accomplish this the dosage of a drug must be properly spaced, but to do this data concerning its absorption and excretion are necessary. With sulfanilamide, absorption from the gastrointestinal tract is complete and a peak blood concentration is reached in 3 to 5 hours;[2] therefore, it is rational to space doses at 4-hour intervals. Rapidity of excretion may necessitate frequent dosage, but a high rate of excretion is of great advantage for freeing the body of the drug when administration has to be stopped on account of toxic manifestations. Rapid excretion is a great advantage in the case of a drug to be used as a urinary antiseptic; a lower blood level of such a drug will give the same urinary concentration as would be obtained with a higher blood concentration of a less rapidly excreted drug. In the case of sulfanilamide, it has been shown that increasing the rate of urine flow by administration of water increases the rate of elimination of the drug:[32] this is probably also true of sulfapyridine and of sulfathiazole. The rapid diffusibility of a drug to all the tissues and fluids of the body and its presence in them in about the same concentration as in the blood is apparently a valuable asset for a good chemotherapeutic agent. This has been shown to be true for sulfanilamide[33] and for sulfapyridine.[24] No complete data are available for sulfathiazole, but it is known that this drug does not pass into the spinal fluid as rapidly as do sulfanilamide and sulfapyridine.[34] Hence, for treating meningitis sulfathiazole is not suitable to replace either sulfanilamide or sulfapyridine.

In conclusion, a brief discussion of the individual variation in the response to these drugs may be given. One finds in clinical reports on the therapeutic efficiency of these drugs statements expressing surprise that no correlation appears to exist between the blood concentration and either the therapeutic response or some symptoms of toxicity such as nausea and vomiting. With the relatively small amount of data available, this is exactly what one would expect from the individual variation of patients and differences in the strains of infecting organisms. Let me illustrate this matter of biological variation by mentioning some experiments on mice.

In Figure 1, the characteristic curve of the acute toxicity of sulfanilamide for mice when given *per os* is shown. One can calculate that

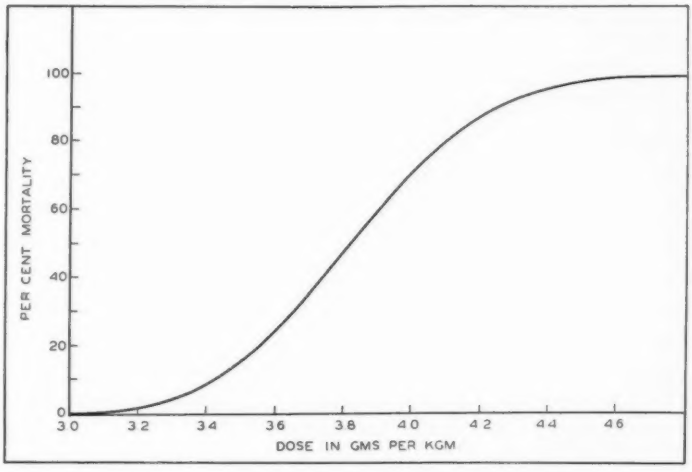

Fig. 1—Characteristic curve of acute toxicity of sulfanilamide for mice, drawn from data of Marshall and Litchfield.[24]

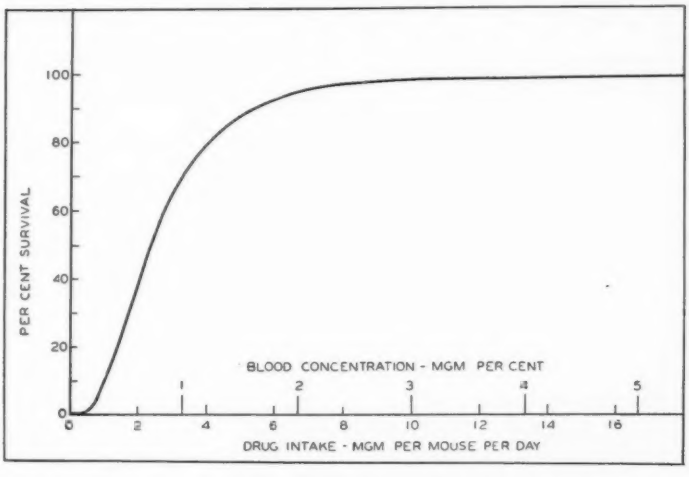

Fig. 2.—Characteristic curve of blood concentration (or drug intake per day) against per cent survival of mice infected with β-hemolytic streptococci and treated with sulfanilamide, redrawn from figure of Marshall, Litchfield and White.[16]

the dose which will kill 1 per cent of the mice is 3.1 gm. per kgm. and that it takes 4.6 gm. per kgm. to kill 99 per cent of the mice. This is not a wide variation. In Figure 2, the characteristic curve of the blood concentration (or drug intake per day) against the percentage survival of mice infected with an invariably rapidly fatal streptococcus infection is shown. While as low a blood concentration as 0.17 mgm. per cent of sulfanilamide maintained for three days will result in a survival of one per cent of the mice, it takes 3.06 mgm. per cent (or 18 times as much) to give a survival of 99 per cent of the mice. This is quite a marked variation. When one considers that sick human beings of widely varying genetic stock infected with different strains or types of an organism are unquestionably much more variable than these mice, it is not surprising but to be expected that in a relatively small series no relation between blood concentration and the toxicity or the therapeutic effect of one of these drugs would be apparent. Also, one would expect that the blood concentration suggested at present for a satisfactory therapeutic response is unnecessarily high for many patients and not high enough for a very few. However, if data from a very large population were analyzed, it is certain that the same continuous variation as seen in mice would be observed.

Let me urge upon the clinicians who are responsible for the treatment of patients with these drugs to try to obtain very accurate data in regard to blood concentration and toxic symptoms or therapeutic effect. If this is done, we shall ultimately be able to analyze such data and possibly improve the treatment of bacterial infections with the sulfonamide group of drugs.

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THE CLINICAL USE OF SULFANILAMIDE AND ITS DERIVATIVES IN THE TREATMENT AND PROPHYLAXIS OF CERTAIN INFECTIONS*

The Wesley M. Carpenter Lecture

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SINCE the original report by Domagk[1] concerning the chemotherapeutic properties of Prontosil, numerous sulfanilamide derivatives have been introduced for the control of bacterial infections. In many countries these derivatives have been launched with but meager preliminary experimental or clinical data, and with claims of therapeutic potency far beyond the real value of the compounds. Fortunately, in the United States, the advent of sulfanilamide was followed closely by the passage of the present Food and Drug Act, and with the exception of the tragic consequences which resulted from the widespread distribution of "Elixir of Sulfanilamide," all sulfanilamide derivatives which have come into the hands of American physicians, have had a firm background of experimental investigation and clinical application. Out of a welter of drugs, American pharmaceutical manufacturers have wisely pressed claims for only three or four sulfanilamide derivatives, and the Food and Drug Administration and the Council on Pharmacy and Chemistry of the American Medical Association have seen fit to recognize only two of them, namely, sulfathiazole and sulfapyridine. This far-sighted policy on the part of all concerned has limited the list of these chemotherapeutic agents to those of proven merit, and tonight it will be our purpose to attempt to give a clinical evaluation of the use of sulfanilamide, sulfapyridine and sulfathiazole in the prophylaxis and treatment of certain bacterial infections.

Shortly after the introduction of sulfanilamide it became evident, from the work of Marshall and his associates,[2, 3] that the rational use of

* Read October 14, 1940 in the Graduate Fortnight of The New York Academy of Medicine.
From the Department of Preventive Medicine, The Johns Hopkins University and the Medical Clinic of The Johns Hopkins Hospital.

this drug called for a knowledge of the factors concerning its absorption, distribution, excretion from the body, and the possible toxic effect which it might have. It quickly became evident that successful therapy with sulfanilamide depended upon the maintenance of certain concentrations of the drug in the blood of the patient under treatment. Later, with the introduction of sulfapyridine and sulfathiazole, and with the discovery that they presented different patterns in respect to their passage through the body from those of sulfanilamide, it has become necessary for the physician to possess a sound knowledge of the clinical pharmacology of these compounds if he expects to obtain the greatest measure of therapeutic success from the use of these agents. The problem as to which one of these compounds it is best to employ in a given infection is a real one. Hence, it is imperative when using these compounds to know of their behavior in the body and to have the latest information regarding their comparative effectiveness in disease—types of data which can be based only upon extensive experimental investigations and sound clinical observations.

More is known about the fate of sulfanilamide in the human body than of either of the other two compounds. This drug, when administered by the oral route, is well absorbed from the gastrointestinal tract, is fairly equally distributed throughout the body tissues and, if kidney function is normal, is promptly excreted. The maintenance of adequate concentrations of sulfanilamide requires a dosage schedule that is on a four hour basis. Sulfapyridine is irregularly absorbed by different individuals, and in the same individual the degree of absorption of this drug from the gastrointestinal tract may vary quite markedly from day to day. As far as information is available, the drug is fairly well distributed throughout the tissues, and, as a general rule, it is conjugated to the therapeutically inactive acetyl derivative in a much higher degree than is either sulfanilamide or sulfathiazole. From data available at the present time, it seems that the liver plays the predominant role in the conjugation of sulfanilamide and its derivatives. Sulfapyridine is excreted more slowly than is sulfanilamide or sulfathiazole and in the urine a high percentage of the excreted drug is generally present in the acetylated form. This conjugated fraction is poorly soluble and tends to crystallize out in the urine.

Sulfathiazole is readily absorbed and when kidney function is normal, is rapidly excreted. It is conjugated by the tissues in about the same

degree as has been noted for sulfanilamide. Data which have been obtained from the administration of the drug in patients whose kidney function is depressed and in whom the rate of excretion of the drug is decreased, tend to show that if sulfathiazole remains in the body for any considerable period of time, its rate of conjugation tends to increase rapidly. This suggests that the reason why sulfathiazole does not ordinarily seem to be converted in large amounts to the acetyl form, is that the drug usually is excreted so rapidly that the conjugating mechanism in the liver does not have time to play its ordinary role. The drug is excreted almost entirely by the kidney, and in the urine definitely less sulfathiazole is found in the acetylated form than is the case with sulfapyridine, again, probably, because the drug is excreted so rapidly. This rapid excretion of the drug makes it difficult at times to maintain adequate concentrations of sulfathiazole in the blood. There is little information regarding the distribution of sulfathiazole in the tissues of the body. However, it is known that sulfathiazole passes over much less rapidly into the spinal fluid than does either sulfanilamide or sulfapyridine.

At the present time the sodium salt of sulfapyridine is available for clinical use, and it is probable that the sodium salt of sulfathiazole will be available in the near future. These compounds are made up in 5 per cent solutions in distilled water, *and should always be administered by the intravenous route*. Immediately after these compounds pass into the blood stream, the sodium ion is split off, thus releasing either sulfapyridine or sulfathiazole as the case may be. From this point on, these drugs behave in the tissues in a manner comparable to that noted when they are administered by the peroral route.

In the selection of the drug for the treatment of a given infection, it is of the greatest importance that the correct etiological diagnosis of the disease process be made by bacteriological cultural methods. However, it is our firm opinion that one should never hesitate to employ these chemotherapeutic agents if one feels that, upon the basis of clinical diagnosis, an infection is present which is known to respond to sulfanilamide or its derivatives. The early use of these chemotherapeutic agents in the treatment of susceptible infections is of paramount importance if prompt, clear cut clinical results are to be obtained. Recently, on the basis of experimental and clinical observations, we have attempted to list the drug of choice in the treatment of certain disease processes. We

TABLE I

THE DRUG OF CHOICE IN THE PERORAL TREATMENT OF BACTERIAL INFECTIONS WHICH RESPOND WELL TO CHEMOTHERAPEUTIC AGENTS

Sulfanilamide	Sulfapyridine	Sulfathiazole
Chancroid	Gonococcal Infections	E. coli Tissue Infection
Gas Gangrene	Arthritis	Gonococcal Infections
Cl. Welchii	Endocarditis	Arthritis
Hemolytic Streptococcal	Female Gonorrhea	Male Gonorrhea
Infections	Male Gonorrhea	Pneumococcal Infections
Abscesses	Ophthalmia	Pneumonia
Adenitis	Pneumococcal Infections	Staphylococcal Infections
Angina (Ludwig's)	Mastoiditis	Carbuncle
Cellulitis	Meningitis	Cellulitis
Empyema	Otitis Media	Osteomyelitis
Erysipelas	Peritonitis	Pneumonia
Impetigo	Pneumonia	Septicemia
Lymphangitis	Sinusitis (Acute)	Urinary Tract Infections
Mastoiditis	Staphylococcal Infections	A. aerogenes
Meningitis	Meningitis	B. pyocyaneus
Miscellaneous		E. coli
Osteomyelitis		S. albus
Otitis Media		S. aureus
Peritonitis		
Peritonsillar Abscess		
Pharyngitis		
Pneumonia		
Puerperal Sepsis		
Scarlet Fever		
Septicemia		
Sinusitis (Acute)		
Tonsillitis		
Ulcers		
Lymphogranuloma		
Venereum		
Meningococcal Infections		
Meningitis		
Septicemia		
Streptococcus Viridans		
Infections		
Abscesses		
Cellulitis		
Meningitis		
Osteomyelitis		
Peridental Infections		
Septicemia		
Trachoma		
Urinary Tract Infections		
Hemolytic Streptococcal (Group B)		
Proteus		

realize full well that there will be a definite disagreement on the part of some physicians concerning our evaluation of the clinical effectiveness of these drugs.

In Table I are listed the drugs of choice in the peroral treatment of bacterial infections which are known to respond well to therapy with sulfanilamide or its derivatives. There can be little doubt on the basis of present knowledge, that hemolytic streptococcal infections produced by organisms belonging to Lancefield's Group A, are best treated by sulfanilamide. The same seems to hold true for chancroid, Welch bacillus infections (gas gangrene), lymphogranuloma venereum, meningococcal infections, and infections (other than subacute bacterial endocarditis) which are produced by Streptococcus viridans, provided that the Streptococcus viridans belongs to the mouth group of these organisms and is not an enterococcus. At the present time it seems best that sulfanilamide be used in the therapy of trachoma and urinary tract infections due to the double zone-β hemolytic streptococcus (Lancefield's Group B) or to B. proteus.

It is to be noted at this point that the use of sulfanilamide in the treatment of gonococcal infections is not recommended. This is based on the observations made by numerous workers during the past two years that sulfapyridine and sulfathiazole are definitely superior to sulfanilamide in the treatment of gonococcal infections. Sulfapyridine, therefore, is an effective agent in the treatment of gonococcal infections, certain pneumococcal infections, and, because it passes readily into the spinal fluid, in staphylococcal meningitis.

There can be little doubt that sulfathiazole is the drug of choice in most staphylococcal infections. As for pneumococcal infections, it seems to be as effective in pneumococcal pneumonia as sulfapyridine, and it produces much less nausea and vomiting in patients. It seems to be as effective in male gonorrhea as is sulfapyridine. From both the experimental and clinical points of view, sulfathiazole appears to be the most effective drug in the treatment of colon bacillary infections of the tissues such as peritonitis, pyelonephritis. In certain urinary tract infections the drug seems to be of considerable value.

In Table II are listed certain diseases in which sulfanilamide or its derivatives have been used, but concerning which not enough reliable data are available to evaluate completely the clinical effects of these drugs. Hence, one cannot recommend these drugs with the utmost con-

TABLE II

DISEASES IN WHICH THE PERORAL ADMINISTRATION OF SULFANILAMIDE OR ITS DERIVATIVES HAVE BEEN REPORTED TO BE OF SOME VALUE

Sulfanilamide	Sulfapyridine	Sulfathiazole
Actinomycosis	Brucella Infections	Ulcerative Colitis
Brucella Infections	Dermatitis Herpetiformis	
Hemolytic Streptococcal Infections (Lancefield Groups B and C)	Friedländer's Bacillus Infections	
Influenza Bacillus Meningitis	Pemphigus	
Lupus Erythematosis	Streptococcus Viridans (Sub-acute Bacterial Endocarditis)	
Malaria	Ulcerative Colitis	
Pemphigus		
Streptococcus Viridans (Sub-acute Bacterial Endocarditis)		
Ulcerative Colitis		

TABLE III

DISEASES IN WHICH SULFANILAMIDE OR ITS DERIVATIVES HAVE BEEN USED WITHOUT SATISFACTORILY DEMONSTRATING THEIR VALUE

Sulfanilamide	Sulfapyridine	Sulfathiazole
Anaerobic Streptococcal Infections	Anaerobic Streptococcal Infections	Anaerobic Streptococcal Infections
Bacillary Dysentery	Common Colds	Influenza
Common Colds	Influenza	Typhoid
Influenza	Paratyphoid Fever	
Non-Hemolytic Streptococcal Infections	Chronic Sinusitis	
Paratyphoid Fever	Tularemia	
Rheumatic Fever (Acute)	Typhoid	
Rocky Mountain Spotted Fever		
Chronic Sinusitis		
Trichomonas Vaginal Infections		
Tuberculosis		
Tularemia		
Typhoid		

fidence that they will accomplish the desired therapeutic results. It is our belief, however, that one should never hesitate at least to try the effects of these drugs in the diseases which have been listed here. The exception to this is malaria in which quinine, Atabrine or Plasmochin are always to be preferred.

In Table III are listed a number of diseases in which sulfanilamide or its derivatives have been tested, and in which there is no good evidence that they have a definite therapeutic value. For this reason their use in these diseases is not advised.

At this point it is important to remember that the rational and reasonable use of these three drugs calls for more thought than just their correct prescription. One must not neglect the patient himself while treating a given infection, and all measures of proven value should be employed when indicated. Of considerable importance is the proper use of surgical procedures when such are advisable. This is especially true in the treatment of the complications of pneumonia, such as empyema, purulent arthritis, the drainage of abscesses occurring in the course of hemolytic streptococcal infections, and especially the incision of purulent foci when sulfathiazole is being prescribed in staphylococcal infections. It has seemed to us that since the introduction of sulfathiazole, possibly a little too much stress has been laid upon the chemotherapeutic activity of this drug in the therapy of staphylococcal infections. On several occasions we have seen individuals suffering from a staphylococcal bacteriemia in whom the administration of the drug brought about a prompt clearing of the blood stream and signs of considerable improvement in the patient. However, in these patients, the temperature and pulse did not come down to normal, and in hopes that further administration of the drug would bring about the desired effect, therapy was kept up over a period of weeks without complete recovery. Finally, sulfathiazole was stopped, and in each instance, hidden and masked foci of infection began slowly to make their appearance. In all instances, multiple surgical procedures were necessary before the patient recovered. These experiences lead us to believe that it is unwise to continue sulfathiazole over periods of weeks in staphylococcal infections, unless there is definite clinical evidence that the patient is constantly improving.

The question of how much fluid should be given to patients who are receiving sulfanilamide or its derivatives is frequently of considerable importance. We have noted repeatedly patients in whom, despite appar-

ently adequate doses of sulfanilamide, it seemed difficult to establish therapeutically active concentrations of sulfanilamide in the blood. In each instance it was found that the patients were receiving such large quantities (4500 cc. to 7000 cc.) of fluids each day that the drug was being excreted almost as rapidly as it was being absorbed. Hence, when patients are receiving sulfanilamide, it is rarely necessary to force fluids beyond 3500 cc. per day.

When sulfapyridine or sulfathiazole is being given to a patient, the administration of adequate amounts of fluids is a prime necessity. This results from the fact that in concentrated urines, the precipitation of acetyl sulfapyridine or acetyl sulfathiazole is more likely to occur and, hence, there is a greater possibility of renal calculi being formed. Therefore, in patients who are receiving either of these drugs, *the urine output should be kept at 1000 cc. or more per day.*

Certain curious beliefs have grown up concerning the diets for patients who are receiving sulfanilamide or its derivatives. Eggs, for instance, are said to be dangerous because of their sulphur content, and because of the possibility that additions of sulphur will facilitate the production of sulphhemoglobin. It is sufficient to say there is not a bit of data available which would lead one to believe that such a superstition has any foundation in fact.

Also, as far as we know, there are no contraindications to the concurrent administration of other necessary drugs, provided that there are definite indications for such prescriptions. The possible exception to this statement is the use of *anesthetic* (not sedative) doses of certain barbiturates in patients receiving sulfanilamide or its derivatives.

Recently, considerable interest has been aroused in the local use of sulfanilamide and its derivatives in the treatment of superficial infections. At the present time our knowledge concerning the true value of these compounds when used locally is meager. However, in our own experience, we have noted that streptococcal ulcers, indolent superficial staphylococcal infections, wounds and burns infected with mixtures of pathogenic microörganisms often clear up promptly when treated locally with powdered sulfanilamide or its derivatives. While no definite information is available regarding optimal amounts of sulfanilamide or its derivatives for local use, it has been suggested[4] that 0.10 gram per square inch of exposed tissue represents an adequate daily dose. On extensively debrided surfaces, this can be sprayed on easily with a pow-

TABLE IV

THE INDICATIONS FOR THE USE OF THERAPEUTIC ANTISERA WITH SULFANILAMIDE OR ITS DERIVATIVES

Gas Gangrene—	Adequate clinical data is not available.
Meningococcal Infections—	No conclusive clinical evidence that the combined use of meningococcal antiserum and sulfanilamide is better than sulfanilamide alone.
Pneumococcal Lobar Pneumonia—	Patients who are severely ill should receive *adequate amounts* of type specific pneumococcal antiserum as well as full doses of sulfathiazole.
Staphylococcal Infection—	Clinical evidence as to the value of combined therapy is not available.
Scarlet Fever—	Patients who are moderately or severely ill should receive *adequate amounts* of scarlet fever antitoxin as well as sulfanilamide.

der atomizer. It is probable that the main value of the local use of sulfanilamide or its derivatives is that it is possible to obtain relatively high concentrations of these drugs in the superficially infected tissues.

At the present time there is some controversy regarding the combined use of specific sera and antitoxins and sulfanilamide or its derivatives. In Table IV are given the indications for such combined uses. As may be seen from the table, only in patients severely ill with pneumococcal lobar pneumonia, or in patients moderately or severely ill with scarlet fever, is there definite information which leads us to believe that combined sero- and chemotherapy is of real value.

The indications for the prophylactic use of sulfanilamide or its derivatives have not been thoroughly established. In Table V we have listed the possible prophylactic uses of these drugs. It seems quite clear at the present time that the administration for a period of 10 days to 2 weeks of adequate doses of sulfanilamide (either perorally or locally) to patients suffering from compound fractures, prevents the development of gas gangrene and probably other types of infection. There also seems to be good reason to believe that if the earliest stages of otitis media (that of the beginning earache) are treated with sulfanilamide or sulfathiazole for a period of 48 to 72 hours, few children will develop purulent infections of the middle ear with the possible resulting train of complications. The observations of Ravdin and Lockwood[5] show quite

TABLE V

INDICATIONS FOR THE POSSIBLE PERORAL PROPHYLACTIC USE OF SULFANILAMIDE OR ITS DERIVATES

Sulfanilamide	Sulfapyridine	Sulfathiazole
Burns	Otitis Media	Burns
Compound Fractures		Peritonitis (Appendectomies, Large Bowel Resections, etc.)
Scarlet Fever Contacts when Dick Test Positive		Urinary Tract Infections (Urological Operations)
Extensive Tissue Injuries		
Accidental		
Operative		
Gunshot Wounds		
Otitis Media (Earache)		
Peritonitis (Appendectomies, Large Bowel Resections, etc.)		
Rheumatic Fever (Quiescent stage)		

clearly that the routine use of adequate doses of sulfanilamide in the postoperative treatment of appendicitis has markedly decreased the morbidity and case fatality rate from peritonitis occurring as a complication of appendicitis. Also the work of Thomas et al.[6] and of Coburn and his associates[7] seems to point to the fact that in the northern half of this country the administration of small daily doses of sulfanilamide from the first of October to the first of June prevents the recurrence of the active manifestations of rheumatic fever in patients suffering from that disease. If these latter observations could be widely confirmed, a most important prophylactic measure would be at our disposal.

The administration of sulfathiazole as a prophylactic agent to patients who are undergoing a resection of the large bowel, or who have the risk of developing peritonitis following operative procedures in the abdomen, has seemed to be most effective in The Johns Hopkins Hospital during the past year. In those patients who have to undergo a resection of the large bowel, the drug is started in doses of 1.0 gram every 4 hours on the afternoon prior to the day of operation and is continued until about the sixth postoperative day.

In Tables VI to X are shown the amounts of sulfanilamide or its derivatives which we believe represent therapeutically effective doses for severe infections due to the hemolytic streptococcus, the meningo-

TABLE VI

THE DOSAGE OF SULFANILAMIDE

Sulfanilamide

A. Severe Infections

Initial dose (oral): 0.10 gram per kilogram of body weight.
Subsequent doses (oral): Total daily dose based on 0.10 gram per kilogram, this to be divided in 6 parts and given Q.4 h. day and night until 7 days of normal temperature have elapsed.

B. Mild and Moderately Severe Infections

Total daily dose based on 0.10 gram per kilogram of body weight, this to be divided into 6 parts and given Q.4 h. day and night until 5 days of normal temperature have elapsed.

C. Hemolytic Streptococcal otitis media, mastoiditis or osteomyelitis present a special problem because these infections involve bone. Sulfanilamide should be continued in moderate doses for at least 10 days after a *clinical* cure has been effected.

coccus, the Welch bacillus, the pneumococcus, the staphylococcus and the gonococcus, and for mild or moderately severe tissue infections in which therapy with these drugs is indicated. When one is prescribing sulfanilamide for the treatment of a severe infection, it is highly important to give a large initial dose in order that a therapeutically efficient concentration of the drug may be obtained rapidly; then sufficient doses of the drug should be given at 4 hour intervals in order to maintain and augment the effect obtained by the initial dose. In general, in severe infections in which sulfanilamide is being employed as the drug of choice, a concentration of the drug of 10 mgms. per cent in the blood should be maintained until a marked clinical improvement in the condition of the patient has been noted. Then the amount of the drug given each day may be slowly decreased. In milder tissue infections in which sulfanilamide is the drug of choice, concentrations of around 5 mgm. per cent of the drug should be maintained in the blood.

On the basis of our present knowledge, it seems as though sulfapyridine and sulfathiazole were equally effective in the treatment of pneumococcal pneumonia, with sulfathiazole generally being the drug of choice because it is less likely to provoke nausea and vomiting. During the febrile period of the pneumonia, the concentration of these drugs should be kept at 4 to 6 mgm. per cent in the blood of the patient.

TABLE VII

THE DOSAGE OF SULFAPYRIDINE AND SULFATHIAZOLE

Pneumococcal Pneumonia

A. Adults

Initial dose (oral) either drug. 4.0 grams.
Subsequent doses (oral) either drug. 1.0 gram Q.4 h. day and night until the temperature has been normal for 72 hours.

B. Infants and Children

Initial dose (oral) either drug, 0.15 gram per kilogram up to 25 kilograms of body weight.
Subsequent doses (oral) either drug. Total daily dose is based upon 0.15 gram per kilogram up to 25 kilograms of body weight, this to be divided into 4 parts and given Q.6 h. day and night until the temperature has been normal for 48 hours.

Staphylococcal Pneumonia

A. Adults and Children

Sulfathiazole is the drug of choice. If satisfactory response is not obtained, increase daily dose by 25 to 50 per cent. Continue until temperature has been normal for 5 days.

TABLE VIII

THE DOSAGE OF SULFATHIAZOLE IN STAPHYLOCOCCAL INFECTIONS OTHER THAN PNEUMONIA

A. Chemotherapy should not be used for boils, mild furunculosis, etc.

B. *Adequate surgical therapy* should *always be used* in conjunction with chemotherapy.

I. Diffuse staphylococcal cellulitis, lymphangitis, etc., in adults

Initial dose (oral): 4.0 grams.
Subsequent doses: 1.5 grams Q.4 h. day and night until the infection has ceased spreading. Then 1.0 gram Q.4 h. day and night for 7 days.

II. Staphylococcal bacteriemia in adults

Initial dose (oral): 4.0 grams
Subsequent doses: 1.5 grams Q.4 h. until the temperature has been normal for 48 hours. Then 1.0 gram Q.4 h. for 14 days. Then 0.5 gram Q.4 h. for another 14 days.

III. Staphylococcal infections in infants and children

The initial and subsequent doses are reduced from those advised for adults in proportion to the weight of the infant or child.

TABLE IX

THE DOSAGE OF SULFAPYRIDINE OR SULFATHIAZOLE IN THE TREATMENT OF GONORRHEA IN THE MALE

Either drug may be used.

First day dose: 3.0 grams, i.e., 0.5 grams at 3 hour intervals six times a day.

Subsequent doses (2nd to 10th days): 2.0 grams per day.

If by the 5th day of treatment a marked improvement in symptoms and signs of the disease has not taken place, shift to the other drug and carry on as outlined above. If a shift in medication has been made and after 5 days marked improvement is not observed, *stop drug therapy* and begin *conservative local treatment.* In any event *do not continue treatment with these drugs beyond 15 days in patients suffering from gonorrhea.*

TABLE X

THE PARENTERAL USE OF SULFANILAMIDE OR ITS DERIVATIVES

Sulfanilamide—To be used only when oral medication is impossible.

Initial dose: 0.10 gram per kilogram of body weight made up in a 1 per cent solution in sterile physiological saline or 1/6 molar sodium racemic lactate.

Subsequent doses: 0.05 to 0.075 gram per kilogram of body weight in a 1 per cent solution, Q. 6 to 8 h.

It is best to give solutions of sulfanilamide by the subcutaneous route. Oral administration of sulfanilamide should be started as soon as possible.

Sodium Sulfapyridine or *Sodium Sulfathiazole*—To be used only when oral medication is impossible or satisfactory concentrations of the drug cannot be obtained by oral administration.

Initial dose: 0.06 gram per kilogram of body weight of the sodium salt made up in a 5 per cent solution of *sterile, freshly distilled water. Do not try to sterilize by autoclaving or boiling.*

Subsequent doses: 0.03 gram per kilogram of body weight of the sodium salt in a 5 per cent solution repeated at about 6 hour intervals.

Always give slowly (10-15 minutes) by the intravenous route.

Oral administration of sulfapyridine or sulfathiazole should be started *as soon as possible.*

In staphylococcal infections, with the possible exception of staphylococcal meningitis, sulfathiazole should always be employed. The exception of staphylococcal meningitis is the result of the observation that *sulfathiazole does not pass over readily into the spinal fluid*. This drug should not be used in the treatment of small boils, mild furuncles, etc., which result from superficial infections with staphylococci, because the risk of toxic manifestations from sulfathiazole is greater than the therapeutic results which may be obtained. *It is also of extreme importance to remember that adequate surgical therapy should always be used in conjunction with chemotherapy in the treatment of staphylococcal infections*, and that when an apparent marked improvement has taken place in a patient severely ill with a staphylococcal infection, if the drug is discontinued too early, a recurrence of the infection is very likely to happen. On the basis of present data, concentrations of 5 to 10 mgm. per cent should be maintained in the blood of patients who are severely ill with staphylococcal infections.

Sulfanilamide and the sodium salts of sulfapyridine and sulfathiazole may be given by the parenteral route. Sulfanilamide is best administered by the subcutaneous route, while sulfapyridine and sulfathiazole should always be given by the intravenous route. When these drugs are administered by these routes, it is always wise to begin the oral administration of the drug as soon as the patient's condition permits, because the per-oral route of administration of these drugs is, in general, the most satisfactory.

In patients whose infections involve bone or bony tissue, the use of these drugs presents a special problem because a recurrence of the infection is liable to take place unless all foci of infection have been eliminated. For this reason again, adequate surgical drainage is important, and in such conditions as otitis media, mastoiditis or osteomyelitis, the administration of sulfanilamide or its derivatives should be continued in moderate doses for at least 10 days after a *clinical cure* has been effected. The non-observance of this rule has been, in a large degree, the cause of the unsuccessful use of these drugs in otitis media or mastoiditis and for the reported masking of the symptoms and signs of these diseases.

One of the curious results of the intensive clinical work that has been done with sulfanilamide and its derivatives and of the publicity derived following such investigations during the past four years, has been to make physicians overcautious about the possible toxic manifes-

TABLE XI

MANIFESTATIONS OF DRUG TOXICITY NOTED IN ADULTS TREATED WITH SULFANILAMIDE, SULFAPYRIDINE OR SULFATHIAZOLE

Reaction	*Sulfanilamide*	*Sulfapyridine*	*Sulfathiazole*
Nausea, vomiting	Fairly common	Frequent	Uncommon
Dizziness	Common	Common	Uncommon
Psychoses*	0.6%, occur early	0.3%, occur early	Rare
Neuritis**	Very rare	Not reported	Rare
Cyanosis	Very common, early and late	Faint, common, early and late	Uncommon
Acidosis*	1.9%, occurs at any time, rare if soda is used	Not reported	Not reported
Fever*	10%, generally 5th to 9th day, may occur 1st to 30th day	4%, generally 5th to 9th day, may occur 1st to 30th day	10%, generally 5th to 9th day
Rash*	1.9%, may take any form, generally 5th to 9th day, may occur 1st to 30th day	2%, may take any form, 5th to 9th day, may occur 1st to 30th day	5%, nodular type common, may take any form, 5th to 9th day
Hepatitis**	0.6%, early or late	Not seen, but reported	Rare
Leukopenia with granulocytopenia**	0.3%, early or late	0.6%, early or late	1.6%, early or late

* Best to stop drug and force fluids.
** Imperative to stop drug and force fluids.

TABLE XI [continued]

Reaction	*Sulfanilamide*	*Sulfapyridine*	*Sulfathiazole*
Acute agranulocytosis**	0.1%, occurs 14th to 40th day, common 17th to 25th day	0.3%, occurs 14th to 40th day, common 17th to 25th day	Not reported
Mild hemolytic anemia	3%, early and late	Rare	Not reported
Acute hemolytic anemia**	1.8%, occurs 1st to 5th day	0.6%, occurs 1st to 5th day	Very rare
Hematuria*	Not reported	8%, generally early	2.5%, generally early
Anuria with azotemia**	Not reported	0.3%, generally 1st 10 days	0.7%, generally 1st 10 days
Hyperleukocytosis*	Generally in presence of acute hemolytic anemia	Generally in presence of acute hemolytic anemia	Not reported
Injection of sclerae and conjunctivae**	Not reported	Not reported	4%, may occur with rash and fever, 5th to 9th day
Purpura haemorrhagica**	Not seen, but reported	Not seen, but reported	Not reported
Ocular and auditory disturbances**	Rare	Rare	Very rare
Jaundice**	With acute hemolytic anemia or hepatitis	With acute hemolytic anemia or hepatitis	With acute hemolytic anemia or hepatitis
Painful joints*	Reported	Not reported	Reported with rash, etc.
Stomatitis*	Rare	Not reported	Not reported
Gastrointestinal tract disturbances*	Bleeding rare, diarrhea uncommon	Rare	Very rare

* Best to stop drug and force fluids.
** Imperative to stop drug and force fluids.

tations of these drugs, while the patients, who read only of the "wonder drugs" in their local press, are clamoring for these agents whenever they have the mildest scratch in their throats or on their fingers. It is our opinion that we, as physicians, have been right in adopting a somewhat cautious attitude towards the use of these drugs, but we also feel that as our experience has grown, it is now possible for the average physician to use these drugs intelligently even in the home, and that he should not worry too much about their possible toxic manifestations.

In Table XI are given the various manifestations of drug toxicity as we have noted them in hospitalized adults. It is evident from this table that the toxic reactions resulting from these drugs are quite frequent, but that those which are dangerous are relatively uncommon. It is our opinion that while it is always advisable whenever possible to utilize every available means of laboratory control in following a patient receiving sulfanilamide or its derivatives, practically all of the toxic reactions associated with the administration of these drugs can be detected by the physician if he exercises careful clinical control over his patient.

Patients who are receiving sulfanilamide or its derivatives for the first time *should be seen at least once a day*, and at this time the attending physician should inquire as to how the patient feels, with special reference to the symptoms of headache, body-aching or malaise, because these symptoms are the precursors of many of the toxic reactions of sulfanilamide or its derivatives. In addition to the inquiries concerning the patient's symptoms, the physician should carefully examine the sclerae for the presence of jaundice, and the conjunctivae for injection or paleness. The presence of jaundiced sclerae with pale conjunctivae probably means that an acute hemolytic anemia is developing. If the conjunctivae are not pale, then the jaundice probably results from liver damage. The occurrence of injected conjunctivae and sclerae, together with smarting and burning of the eyes occurs as a toxic manifestation only in the course of sulfathiazole therapy. As an added check to the conjunctival examination, the oral mucous membrane should be looked at, and inquiry should be made as to whether or not the patient is developing a sore throat. If the patient has been treated with sulfanilamide or one of its derivatives for more than a week, and while under treatment develops a moderately severe sore throat, this may indicate the beginning of an agranulocytosis, because, if he is being treated adequately, it would be quite unlikely that he would be getting a hemolytic strepto-

coccal sore throat. The skin of the body should always be looked at carefully for the presence of rash, because frequently in the early stages of such a toxic manifestation, the patient or his attendants will miss the fine macular eruption in the skin. At this point we can stress the importance of keeping patients who are receiving sulfanilamide or its derivatives out of the direct rays of the sun and away from sources of ultraviolet light, because there is no doubt that a certain number of patients who develop a skin rash in the course of therapy do so because of a photosensitization. Any patient receiving these drugs should stay out of the sun until three days after he has stopped taking the drug, or until after the time when the drug has been completely excreted.

The temperature should always be taken in order to detect whether drug-fever is present, and if the patient says he has been having chills, and at the time that it is taken, the temperature is normal, arrangements should be made to have it taken frequently during the next 24 hours in order to determine whether or not fever is present.

Inasmuch as sulfanilamide does not directly damage the kidneys, no special precautions have to be observed in respect to the urine of patients who are receiving sulfanilamide, but it is very important that the urine of patients who are receiving sulfapyridine or sulfathiazole be looked at when freshly voided, and that the total urine output be measured daily. This can be done easily by the patient's attendants or family and a urine volume of at least 1000 cc. per day should be maintained. If the urine output starts to drop and the fluid intake has been properly maintained in patients who are receiving sulfapyridine or sulfathiazole, it is very good evidence that the drugs are damaging the kidneys either due to a direct toxic effect upon the kidney epithelium or as a result of mechanical blockage by aggregations of acetyl sulfathiazole or acetyl sulfapyridine crystals. However, in the face of a normal urine output, the presence of these crystals in the voided urine should never be considered an indication for stopping the drug. The appearance of gross blood in the urine at any time in the course of therapy with these drugs constitutes a good reason for stopping therapy.

Thus, it can be seen that, with the possible exception of leukopenia or agranulocytosis, all of the toxic reactions resulting from the administration of these drugs can be discovered if the patient is kept under careful clinical observation during his course of treatment, and for this reason it has proved highly practical for careful physicians to employ

these drugs in patients who are being treated in their homes. If toxic manifestations appear, these drugs should be discontinued and fluids should be forced vigorously in order to eliminate them from the system as rapidly as possible.

Finally, it must be kept in mind that if a patient has previously had a rash, drug-fever, hepatitis, leukopenia, acute hemolytic anemia, injection of the sclerae and conjunctivae, diarrhea or purpura hemorrhagica in the course of therapy with sulfanilamide or its derivatives, he is very likely to have a second, earlier and more severe toxic reaction if sulfanilamide or one of its derivatives is administered a second time. For this reason one must always ask a patient whether or not he has taken one of these drugs previously with a resulting toxic reaction, before prescribing any member of this series for a second time. If such a history is elicited, it is best to give a small test dose of the drug (0.15 to 0.3 gram) and then observe the patient for acute toxic manifestations over a period of 12 hours before beginning the course of therapy. If none is observed, treatment with the drug may be cautiously begun and continued, with a constant watch being kept for possible toxic manifestations.*

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* Certain of the dosage schedules which are given in this report are based upon those recommended by the Advisory Committee on Chemotherapeutic and Other Agents of the National Research Council.

ELECTROPHORETIC ANALYSIS AND THE CONSTITUTION OF NATIVE FLUIDS

Harvey Lecture, October 19, 1939

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RECENT years of research in the borderland between chemistry, biology, and medicine have given us many new examples of a fact known since long ago; namely that the fundamental processes of life are intimately connected with chemical substances of large molecular weight. Only such substances are capable of the great variation and specificity which are considered to be characteristic of life itself. Among these substances proteins should be mentioned first, but we know now that large carbohydrate molecules also are capable of considerable variation in structure and biological properties. The nucleic acids present in the cell nuclei and in many virus proteins are probably in many cases large molecules. It is true that a considerable number of substances essential for life are of comparatively simple structure like the vitamins and many hormones. On the other hand, there is increasing evidence that these depend to a considerable extent for their activity on their interaction with large molecules, especially with proteins. Without proteins no life is possible. No wonder, therefore, that there has been an enormous concentration of efforts by scientists all over the world to contribute to our knowledge of the structure and reactions of these and other large molecules. The results gained so far, and particularly some of the more recent developments in this field, have been very encouraging as some of the most important processes in living organisms have been shown to depend upon specific proteins, which can be isolated in what seems to be pure state, as, for example, some of the enzyme proteins. Also there is considerable evidence that antibodies in immune sera are proteins, and the recent researches of Stanley and others have demonstrated that the same is true for certain viruses. Such fundamental phenomena as muscle contraction and growth have been interpreted as

protein reactions, and interesting attempts have been made to correlate morphology and chemical structure.

While watching the developments in this field of research during the last few years one is struck by the diversity in the modes of attack on the problems. Structural organic chemistry is, of course, still the basis for this research, as it was in Emil Fischer's time, and for proteins the recent work of Bergmann, Niemann, and their collaborators has brought us much new information along these lines. Many of the new contributions have also come from physical chemistry, and I think I am justified in mentioning here especially the name of my countryman and teacher, The Svedberg. But also physics, x-ray crystallography, spectroscopy, immunology, genetics, medicine, and even pure mathematics have contributed to the new advances. There is hardly any field in modern science where research is carried on at so many frontiers simultaneously.

I wanted to emphasize this fact particularly, as I am going to present to you tonight the development of and the results gained mainly by only one method, namely, by electrophoresis, since the president of the Harvey Society has been kind enough to ask me to tell you something about the recent developments in this field. Here as well as in other fields of protein chemistry the most significant results have been obtained by combining evidence gained by a number of different methods.

Electrophoresis, that is the migration of colloidal particles in an electric field, has been known and studied by physical chemists for the past 100 years. It first became of significance for biochemistry, however, in 1899, when Sir William Hardy[1] observed that proteins show this phenomenon in a highly characteristic way and that their migration velocity depends upon the acidity of the medium. Among the early work in this field mention should be made especially of the investigations by Michaelis and his collaborators, which demonstrated the value of the method for the characterization of enzymes and proteins by their mobilities and their isoelectric points.

The most direct method of studying electrophoresis, namely the observation of the migration of individual particles in a solution with the microscope or the ultramicroscope is, unfortunately, very limited in its application, as most of the substances which are of interest have such a small molecular or particle size that they are beyond the resolving power of any microscope. The observation of electrophoretic migration must, therefore, depend upon measurements of the movement of a layer

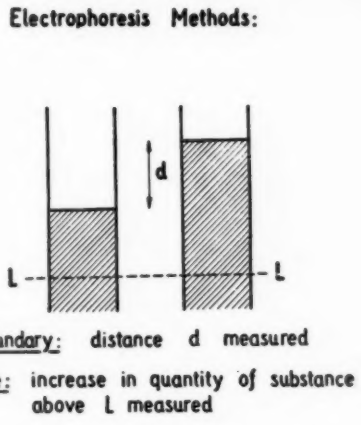


Fig. 1—Diagram of electrophoresis methods.

of the solution of the substances in the electrophoresis tube. For this purpose two different methods have been used (Fig. 1); one, the transference method, depends upon the determination by chemical or biological analysis of the increase or decrease of the amount of substance above a fixed level in the tube, when a known amount of electricity is sent through, the other, the moving boundary method, measures the distance traveled by the boundary between the solution and a supernatant medium of the same composition with respect to the electrolytes. The former method has been used particularly for studying enzymes; the latter is especially convenient for colored materials. Both methods are, of course, in principle identical with those used by the physical chemists for measuring migration velocities of ordinary ions.

Svedberg, in his first studies of the ultracentrifugation of proteins, made use of their strong absorption in ultraviolet light to observe their sedimentation. The application of the same technique to observe the moving boundaries in the electrophoresis tube was tried by Svedberg and myself in 1926 with some success.[2] In this way the moving boundary method could be applied also to colorless substances. The improved possibilities of observing what was happening in the electrophoresis tube showed, however, that the electrophoretic migration suffered from several disturbances. A detailed study of these phenomena and a description of an improved apparatus was published in 1930[3] together with measure-

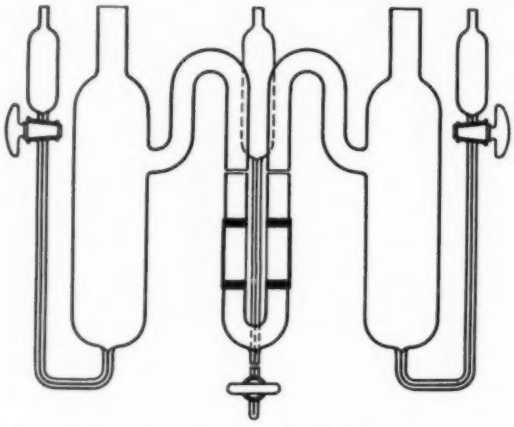


Fig. 2—Electrophoresis apparatus for observation of the migration by photography in ultraviolet light.

Fig. 3—The separation of egg albumin and Bence-Jones protein observed by ultraviolet light photography.

ments on a number of comparatively well-defined proteins (Fig. 2). One important improvement in the new type of apparatus was the introduction of large volumes of buffer solution in the electrode vessels to protect the protein boundaries from being reached by the disturbances which inevitably occur at the electrodes. Figure 3 shows a series of successive photographs of one limb of the tube when a mixture of egg albumin and Bence-Jones protein was studied. It demonstrated the possibility of using the method as a tool for studying unknown mixtures, and perhaps also the application for separation purposes. Of course, the most obvious importance of electrophoresis is the determination of

mobilities and isoelectric points for the purpose of characterization, and, if possible, for the calculation of the charge, and a large number of determinations of this kind have already been made in Upsala as well as in other laboratories. However, the application of the method for the study of mixtures and for preparative purposes is of particular importance for some of the problems I am going to discuss this evening.

The electrophoretic separation of the two proteins in Figure 3 suggests a more general application, but it is well known to everybody working in this field that in most cases great difficulties are encountered. No doubt the possibilities of electrophoretic separation have been realized for a long time. These difficulties may, however, be one reason why procedures of this kind have come into use only slowly in the biochemical laboratories. Another reason may be that the particularly great value of gentle methods for isolation of biologically important large molecules has been realized only lately. The successful use of adsorption, chromatographic analysis, ultrafiltration, and ultracentrifugation in the study and fractionation of mixtures of large molecules as they occur in nature has been one of the most striking and important recent developments in the methods of biochemistry. Early attempts to make use of electrophoresis for similar purposes (purification of pepsin) were made by Ringer,[4] and the method also soon found certain applications in industry (purification of gelatin). More recently Theorell, who has contributed much to the improvement of electrophoresis methods, was able to isolate Warburg's yellow enzyme this way,[5] and similarly du Vigneaud and his collaborators obtained a considerable fractionation of the hormones of the pituitary gland.[6, 7]

During the last few years in the Upsala laboratory several improvements in the technique of electrophoresis have been made[8] which considerably increase the efficiency of the separation and appear to widen the scope of the method to a large extent. The immediate reason for these attempts was the desire to study sera and a number of other native fluids. These are mostly characterized by a comparatively high conductivity on account of the large amount of salts present. Also in other cases large amounts of electrolytes are necessary to ensure a disturbance-free migration. Perhaps the greatest difficulty in electrophoresis is caused by the heat generated by the current. In solutions of considerable salt concentration this will give rise to convection currents which will stir up the solution in the tube and may entirely spoil the boundaries.

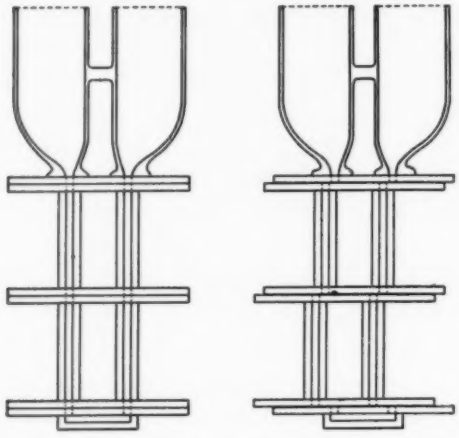


Fig. 4—The assembled U-tube of the new electrophoresis apparatus.

Fortunately, there is a comparatively simple way of eliminating this difficulty to a large extent. Water and solutions in water have a density maximum near +4° C., that is, the density variation which accompanies a rise in temperature and which is responsible for the convection will be negligible if the electrophoresis tube is kept in the neighborhood of this temperature. The apparatus is immersed in a bath which is kept about one degree above zero, and with maximum current the temperature in the tube rises to about the right value. The electrophoresis tube itself is built up of glass cells of rectangular cross section (25 × 3 mm.) with optically plane walls (Fig. 4). These cells can be made to slide over one another, thus separating the contents of the U-tube into five samples (right in Fig. 4). The sample under investigation is filled into the bottom and the lower of the middle compartments, the latter is moved out to one side, and the upper part is filled with a buffer solution of the same composition as that of the sample (usually they have been dialyzed against each other before filling the cells). By sliding the lower middle cell back into alignment with the others the boundaries are formed. The necessary back and forth movements of the cell are performed with small air pressure pumps, shown right and left in Figure 5, which also shows the U-tube mounted in a metal stand. Figure 6 is a diagram of the complete apparatus immersed in the bath (the cooling

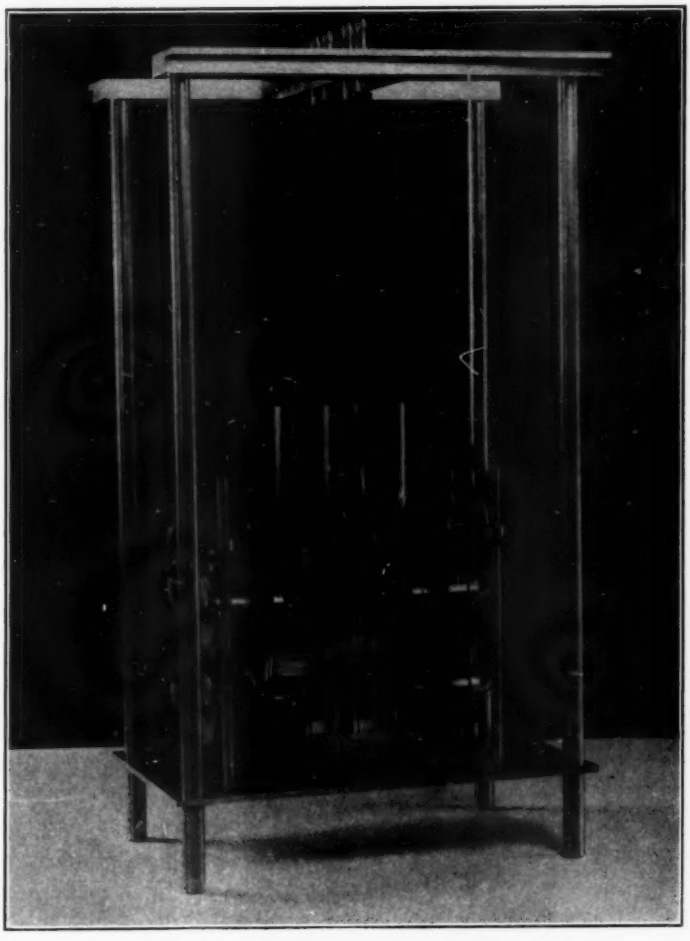


Fig. 5—The U-tube mounted in a metal stand.

coil is seen on the right). The U-tube has been connected to electrode tubes (as in the apparatus in Fig. 2), which contain reversible silver-silver chloride electrodes (not shown in the figure).

The apparatus as now described may be used for migration determinations by the transference method or for separation purposes. Its capacity is 10-11 cc. After a sufficient migration or separation has been obtained the U-tube is divided up, as in Figure 4 (right), so that the

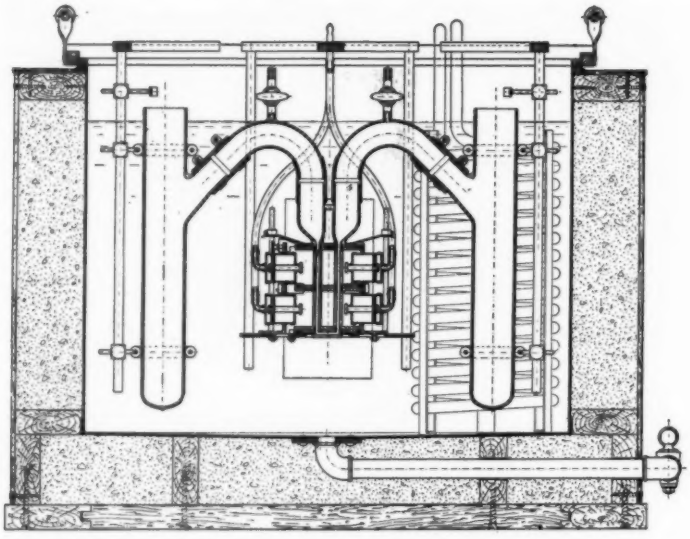


Fig. 6—Complete apparatus in low temperature bath.

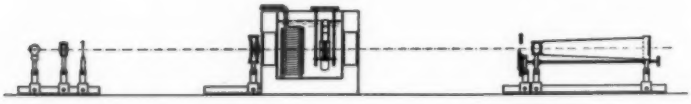


Fig. 7—Optical arrangement for observation of electrophoresis by the schlieren method.

different portions may be obtained separately for analysis or for further investigation. However, the optical observation of the migration has many advantages, especially when dealing with unknown mixtures, and also for convenient mobility measurements. Instead of the ultraviolet absorption, a method depending upon refractive index was introduced which proved to be much superior both in accuracy and convenience. This method, the Toepler schlieren method, has found extensive use for detecting small inhomogeneities in refractive index, for example in testing optical glass, and can be made extremely sensitive. The arrangement used for electrophoresis observation is shown in Figure 7. The apparatus is seen mounted in the bath, and the cells can be observed or photographed with the camera (right). The illumination

Fig. 8—Schlieren photographs of the migration of egg albumin.

system consists of a horizontal slit (left) an image of which is projected onto the camera lens by a large objective close to the apparatus. An adjustable horizontal knife edge at the camera lens will normally admit all light which passes through the electrophoresis tube into the camera. If, however, there is a refractive index gradient somewhere in the tube, as is the case at each boundary, light passing through this level will be deviated downwards and screened off by the knife edge so that the corresponding part of the image of the U-tube in the focus of the camera will receive no light from the illumination system. The boundaries will, therefore, appear as horizontal black bands in this image, and may be observed visually on the screen or photographed, making an exact determination of their position in the tube possible. The width of the schlieren bands depends upon the homogeneity of the migrating substance and, to a certain extent, on the setting of the diaphragm.

Figure 8 shows a number of exposures obtained in one of the first experiments (migration of crystalline egg albumin). For the subject of this lecture it is particularly important to notice the good definition of the position of the boundary, which greatly improves the possibility of studying mixtures. This was confirmed by actual experiments, using a number of artificial mixtures of different proteins. It was also obvious that by using the optical method as a guide, a separation of samll quantities could be effected, the contents of the electrophoresis tube being divided into samples at the end of a run in the way shown in Figure 4 above.

I have described these improvements in the method to you in some

detail as I believe that in this field it is extremely important to use a satisfactory experimental technique. Electrophoresis experiments badly conducted will not only give incomplete or uncertain information; they may give very definite information which is entirely false. On the other hand, it is obvious that one does not take the trouble to concentrate his efforts on the improvement of a method unless there is some reason to believe that it will be of importance for the kind of research we are engaged in. I have already emphasized the importance of the application of gentle methods in work with large molecules obtained from native material, and I should like to add a few more words on that point. When a substance is obtained from such material, for example, by extraction and precipitation with salts or with organic solvents, the question which always occurs is: Does this substance also occur in the native material, or have we modified its properties in any way by our method for its isolation? Any one who has done work of this kind will, I believe, recognize the difficulty. To take an example, there is no doubt if we precipitate a protein in this way, containing some carbohydrate or some lipids or nucleic acids, that all the substances obtained occur in the original material; but the question is; Do they occur together in mutual combination or are they free from each other? The importance of that question has been gradually realized in biochemistry as we have learned that even very weak forces acting between molecules may have a profound influence on their chemical and biological properties. We know that a toxin is neutralized by its specific antitoxin, forming a very unstable complex which may even be partially dissociated by dilution of the solution, and similar conditions seem to hold for the neutralization of viruses by specific substances. We also know that prosthetic groups, of different kinds, nucleic acids and carbohydrates may modify the biological properties of proteins in an essential way. We have every reason to believe that life itself, to a large extent, must depend upon very delicate structures, as yet unknown to us but characterized by the linking together of the various essential molecules in a highly specific and characteristic way. It is conceivable that such structures would be destroyed by many of the drastic procedures usually employed in chemical work. With gentle methods there is at least some chance that the constituents isolated will correspond more closely to those in native conditions. In this connection it may be of interest to observe that already we have some indication that materials obtained by such methods

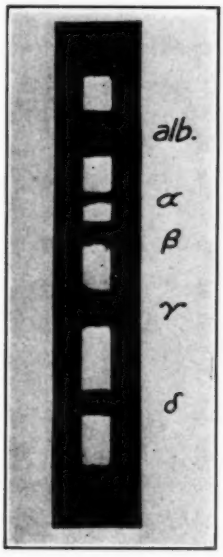


Fig. 9—Electrophoresis of horse serum.

or studied *in situ* in the organism show regularities which may not be found with degraded material. I am thinking especially of the molecular size and homogeneity of proteins as found by Svedberg and his collaborators and of the results obtained by Astbury and others by x-ray methods, showing that crystalline, or at least semicrystalline, structures occur frequently in organisms.

I have already mentioned that the immediate reason for our attempts to improve electrophoresis methods was our intention to study blood serum in this way. The present views of the constitution of this fundamentally important fluid vary a great deal, depending mainly upon the methods used for its study and, in particular, its fractionation into protein components. It has been assumed by some that serum contains essentially only one protein molecular species, the "serum molecule" (Block[9]), and by others a large number of different proteins which cannot be distinguished sharply on account of their mutual interaction (Sörensen[10]). By ultracentrifugation two main components were found to be present and were identified as albumin and globulin.* Our first

* As found recently by Pedersen, molecular size is not a suitable property by which to distinguish between albumin and globulin as part of the serum globulin may assume the molecular weight of albumin.

experiments using the earlier type of apparatus (Fig. 2) with serum globulin, which had been found homogenous with respect to its molecular size, gave definite evidence that it was inhomogenous electrophoretically, but the resolving power of the method was too low to give any more detailed information. The first experiment with the improved method (Fig. 9), obtained with horse serum showed the presence of a number of distinctly different components which were isolated by separation.[8, 11] As only the fastest of these was not precipitated by half saturation with ammonium sulphate, this substance was identified as albumin and the rest as globulins, and these were named α, β, and γ globulins. The δ-boundary in Figure 9, which remains at the position of the original whole serum boundary and does not migrate, does not represent a component but depends upon an inhomogeneity in the conductivity of the medium which is to be expected in concentrated protein solutions and may be accounted for theoretically (see for example Henry and Brittain[12]). Similar diagrams have been obtained with sera from other species, including human beings. Human plasma was shown by Stenhagen to contain an additional component which was identified as fibrinogen since it disappeared on clotting.

In order to study the chemical characteristic of these components it was necessary to isolate them in large quantities; the method used for this purpose will be described below. The results obtained by Blix and Svensson (to be published shortly) for the lipoid contents are of particular interest.

Globulin β: 4.6—12.7 per cent cholesterin, 0.31—0.46 per cent lipoid phosphorus
Globulin γ: 0.71—0.86 per cent cholesterin, 0.03—0.05 per cent lipoid phosphorus
(in normal human sera)

These figures show that the β-component has a high affinity for phosphatides or that the phosphatides move with a mobility close to that of β-globulin. The fat globules in serum move with the β-boundary. They are probably surrounded by an adsorbed layer of β-globulin. In horse sera the boundaries are considerably less distinctly separated than in human, rabbit, and cow sera. Even for these, however, it does not seem likely that each component represents a definite chemical individual, especially since the γ-boundary is mostly rather broad, and also for the others it seems more reasonable to assume that they are groups of similar proteins with each group well distinguished from the others. It is quite likely that a higher resolution will split the components further. We

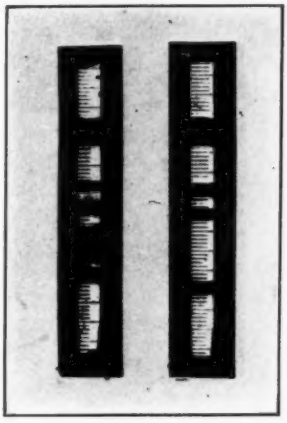


Fig. 10a—Electrophoresis of antipneumococcus horse serum.

plan to increase the separation by using a cell of twice the normal length to study this problem further. Until this has been done and until protein components obtained from serum by other methods have been compared with ours, I think it is too early to try to give a more complete description of the constitution of serum, not to mention an agreement upon a suitable terminology.

It is obvious that a vast field remains to be explored by these methods: for example, a comparative study of the sera throughout the animal kingdom, the development of the serum constituents in an individual with age, changes in pathological cases and during immunization. So far a number of exploratory investigations have been made both in Upsala and elsewhere, and I shall report on some of the results. It should be remembered, however, that the full significance of the results will not be understood until more is known about the normal sera in a few well-defined cases, especially regarding the chemical characterization. The fact that in these diagrams only the mobility is used for the definition of the components makes the evidence obtained somewhat limited in a complicated mixture like serum, as it seems quite likely that different constituents may have almost the same mobility at the pH commonly used for these studies. Figure 10a (Tiselius and Kabat[13]) shows the electrophoretic patterns obtained with a horse antipneumococcus serum before (left) and after (right) removal of the antibody by precipitation

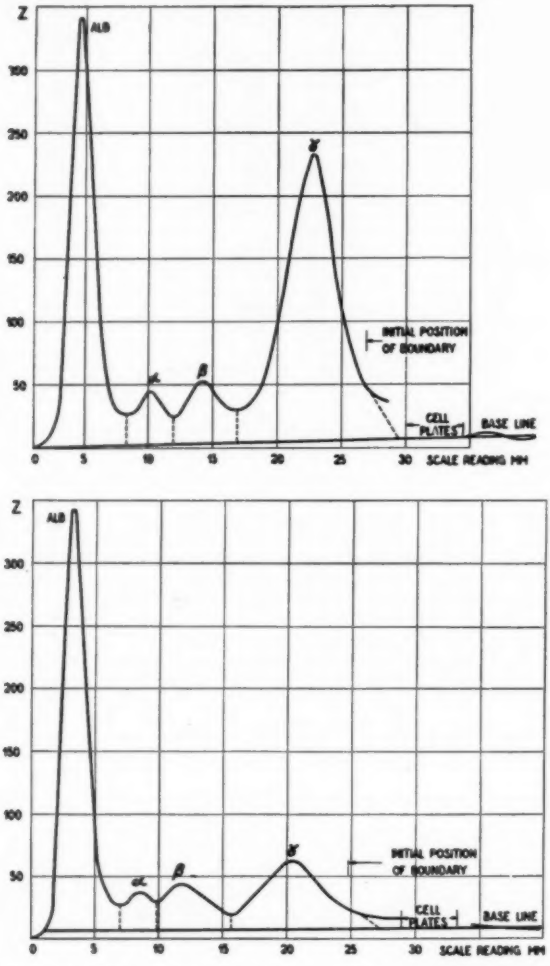


Fig. 10b—Electrophoresis of anti-egg albumin rabbit serum (upper curve) and normal rabit serum (lower curve).

with the homologous polysaccharide. In this case the antibody globulin, therefore, appears to be electrochemically distinct from the other globulins. This is in agreement with the finding[14, 15] that its molecular weight is much higher than that of normal serum globulin. With rabbit antisera, however (Fig. 10b), the antibody in the cases studied showed up as an increase in the γ-fraction, not as a separate boundary and had the

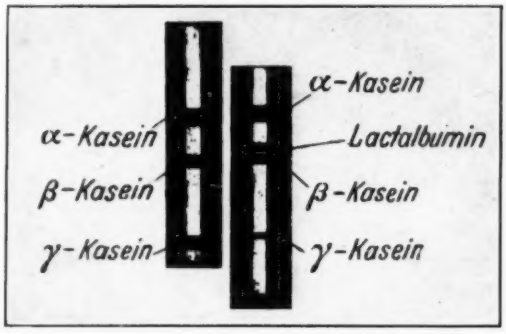


Fig. 11—Electrophoresis of casein (left) and of milk (right).

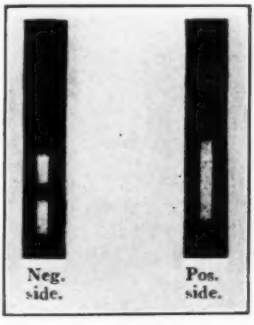


Fig. 12—Electrophoresis of crystalline lens extract (after 35 min.).

molecular weight of normal globulin. This is also the case with some horse antisera when the horse has been under immunization for a long period (Tiselius and Kabat[13]) as has recently been confirmed by Moore, van der Scheer and Wyckoff.[16] It should be emphasized, however, that the normal γ-globulin shows inhomogenous migration and a low mobility so that even a marked difference between normal and antibody globulin may not show up with the method described.

Another case of electrophoretic analysis of native fluids is shown in Figure 11, from experiments by Mellander.[17] It shows the electrophoretic pattern obtained with dialyzed milk (right) as compared to that obtained with a solution of casein, purified according to Hammarsten. Three casein components (α, β, γ) were observed in both cases, and the milk

Fig. 13—Electrophoresis of vitreous body extract (after 35 min.).

diagram also shows the presence of lactalbumin. An investigation of a water extract of the crystalline lens (Hesselvik[18]) showed the presence of two proetins (Fig. 12) which could be identified as α-and β-crystallin first isolated by Mörner.[19] Hesselvik also studied filtered vitreous bodies (Fig. 13) which demonstrated the presence of serum albumin, γ-globulin and a mucoprotein: hyalomucoid, moving faster than the albumin. Many other cases have been studied (see for example Hesselvik[20]), but these may be sufficient to demonstrate the application of the method in the study of mixtures for determining the number of components present and characterization of these components by their mobilities.

Considerable refinements have recently been introduced in the method of optical observation of electrophoresis, similar to those used in the work with the ultracentrifuge. The schlieren diagrams just demonstrated are sufficient to localize the components but do not yield all the information which may be gained from a more complete knowledge of the concentration distribution in the electrophoresis tube, unless a large number of observations are made with different settings of the schlieren diaphragm. A very accurate but somewhat laborious method for studying the concentration gradients was worked out by Lamm several years ago[21] and has found extensive use in diffusion and ultracentrifugation work. It has also been used to some extent in electrophoresis work by several investigators (Annetts;[22] Tiselius and Kabat;[13] Tiselius and Horsfall[23, 24]). Lately, similar methods, also depending

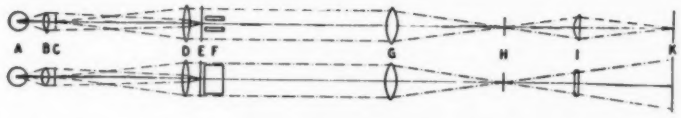


Fig. 14—Diagram of the Philpot-Svensson method for observation of concentration gradients. Upper half of diagram shows arrangement viewed from above, lower half from the side. *A*, lamp; *B*, condensing lens; *C*, slit; *D*, schlieren lens; *E*, screen; *F*, electrophoresis cell; *G*, camera lens; *H*, tilted slit; *I*, cylindric lens; *K*, photographic plate or screen.

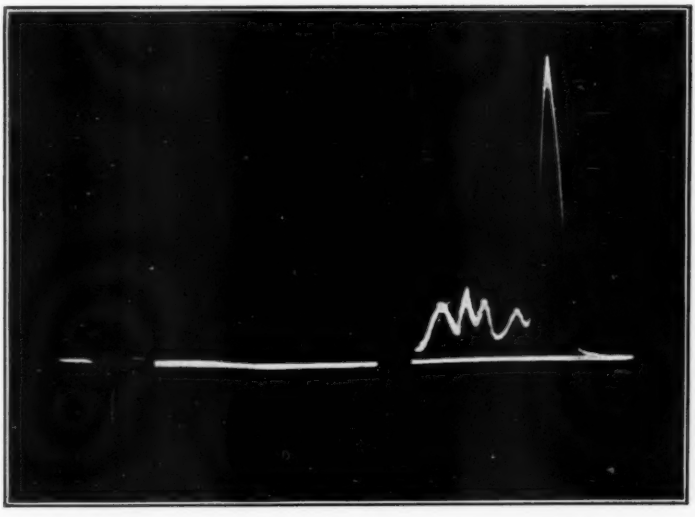


Fig. 15—Diagram of the electrophoresis of human serum obtained by the Philpot-Svensson method.

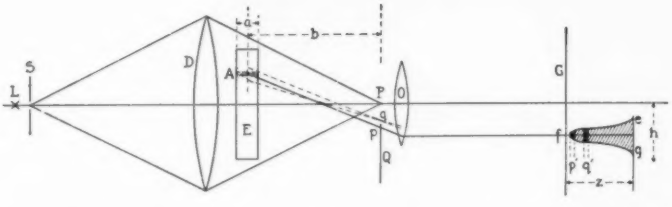
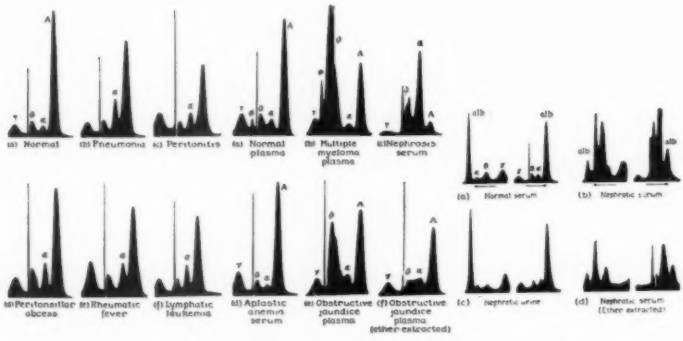


Fig. 16—Diagram of Longsworth's "schlieren scanning" method. *L*, lamp; *S*, horizontal slit; *D*, schlieren lens; *E*, electrophoresis cell; *Q*, schlieren diaphragm, the movement of which is mechanically coupled to the movement of the plate, *G*, at right angle; *O*, camera lens.



Figs. 17, 18, 19—Electrophoresis diagram of normal and pathological sera and plasmas (from Longsworth, Shedlovsky and MacInnes [29]).

upon refractive index although considerably simplified, have been worked out by several investigators (Philpot;[25] Longsworth;[26] Pickels; Andersson;[27] Svensson [28]). Figure 14 shows the arrangement by Philpot, somewhat modified by Svensson, now in use in the Upsala laboratory, and Figure 15, a diagram obtained from human serum by this procedure. Such curves are obtained directly in the focus of the camera and may be observed during the experiment and photographed. Each peak corresponds to a schlieren band, and the area under each peak is proportional to the amount of the corresponding component. Thus, a quantitative electrophoresis analysis can be made, and a much more detailed comparison between diagrams is made possible. The curves may demonstrate the presence of insufficiently separated components in a much more sensitive way than the simple schlieren photographs. Longsworth's arrangement is shown in Figure 16. It depends essentially upon a mechanically coupled simultaneous movement of the photographic plate in the focus of the camera, and the schlieren diaphragm in front of the camera lens. The method has already been applied to a detailed investigation of a number of pathological human sera and plasma (Longsworth, Shedlovsky, and MacInnes[29]). Figures 17, 18 and 19 show some of the results obtained. It was found that normal sera showed good agreement and gave reproducible results. A group of pathological sera was characterized by an abnormal increase in the α-globulin (Fig. 17). Some similar observations had been made by Blix[30] using Lamm's method for recording the diagrams. Figure 18 shows some additional

diagrams, obtained by the first mentioned authors, where still more striking changes can be seen, involving an increase of the β-fraction (or a fraction of the same mobility) and in the case (c), a striking decrease in the albumin and increase in the β-globulin. Of the diagrams in Figure 19, obtained from a case of nephrosis, a comparison of (b) and (c) with (a) shows how the pathological urine proteins resemble those in normal serum, whereas the serum itself differs strikingly. Ether extraction in this and other cases (Fig. 18) shows that part of the abnormal increase in the β-fraction is due to ether soluble material, probably lipoids.

It it evident that the electrophoresis method is able to yield much more detailed information on the constitution of pathological sera than the usual precipitation methods. It will be of great interest to correlate the results with those recently obtained by Kendall,[31] using immunological reactions for the characterization of the serum proteins. It seems that still more detailed information might be obtained if the resolving power of the method could be increased as some of the peaks show a tendency to split up further. With this aim in mind, a cell of twice the usual length is now being constructed especially for serum work.

In the diagrams just shown (Figs. 17, 18, 19), which were obtained from the descending side, a disturbance is observed at the β-boundary, showing up as a very narrow and sharp peak. The nature of this is not yet clear. Possibly some precipitation takes place on account of the removal of albumin and α-globulin on this side from the β-boundary, indicating that the presence of these proteins is necessary to keep some of the serum constituents in an unchanged state. It is a general experience from our work with serum that the β-component is the most unstable of all the serum constituents and is very difficult to isolate.

It is evident that electrophoretic analysis should be capable of giving some information also regarding reactions taking place between components in mixtures. It has been possible to demonstrate that in serum the pigment, bilirubin, is attached to the albumin (Bennhold;[32] Pedersen and Waldenström[33]). Smetana has shown that a number of dyes combine with denatured but not with undenatured proteins (unpublished work). Figure 20 shows an electrophoretic diagram obtained with a mixture of egg albumin and anti-egg albumin in the inhibition zone (excess of egg albumin, precipitate dissolved). The fastest boundary is the excess egg albumin, the slower is the complex between egg albumin and antibody globulin, moving at a rate intermediate between that of each

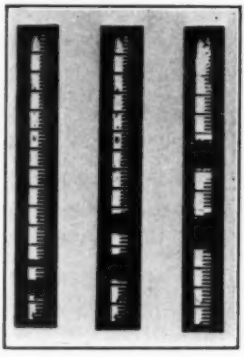


Fig. 20—Electrophoresis of a mixture of egg albumin and anti-egg albumin.

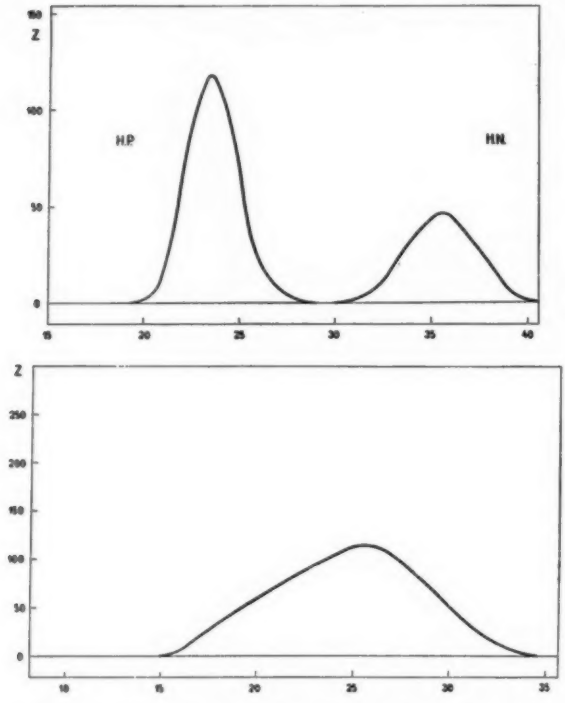


Fig. 21—Electrophoresis of a mixture of *Helix pomatia* and *Helix nemoralis* hemocyanins before (upper curves) and after (lower curves) cross combination by reversible dissociation-association.

component alone (Tiselius and Kabat, unpublished work). Figure 21 gives curve diagrams obtained from a mixture of hemocyanins from two different snails: *Helix pomatia* and *Helix nemoralis*. The upper diagram was obtained after mixing the two proteins, the lower after producing a reversible dissociation-association by shifting the pH to alkaline reaction and then bringing it back to the original value. This is sufficient to cause a formation of a series of cross compounds, a number of mixed molecules with properties intermediate between those of the two original native proteins (Tiselius and Horsfall[23]). Such cross combination, however, seems likely to occur only with the largest protein molecules, which are known to dissociate easily, and, moreover, only with biologically related substances.

In the course of preparation of pure proteins from native material the electrophoresis diagrams are often very helpful as a guide, to show how the preparation proceeds and the degree of homogeneity of the resulting products. This application reminds one of the use of spectrum analysis in chemistry for the detection and isolation of new substances. The spectrum-like appearance of the schlieren photographs is actually more than a superficial analogy. However, in a number of cases the preparative separation by use of electrophoretic migration itself has sometimes proved extremely useful, especially for the isolation of very unstable compounds and for the removal of impurities which are difficult to get rid of by other methods, particularly precipitation. It has been possible to separate the serum components this way, except perhaps for the β-globulin (Tiselius;[11] Tiselius and Svensson, to be published shortly), and to isolate carbohydrates from proteins in tuberculin preparations (Seibert, Pedersen, and Tiselius[34]), just to mention two examples. In enzyme chemistry this method seems to be of particularly great importance as shown especially by Theorell in his purification of Warburg's yellow enzyme and recently also of cytochrome c.[35] It has often been found that traces of impurities which still remain after other purification methods have been applied can be removed by electrophoresis (see for example also the purification of heparin by Wilander[36]). This circumstance may become of particularly great importance in the preparation of substances for immunological work when a trace of an impurity of a large molecule may be of decisive importance. It is a remarkable characteristic of the method that it is almost independent of the relative proportions between the components; the difference in

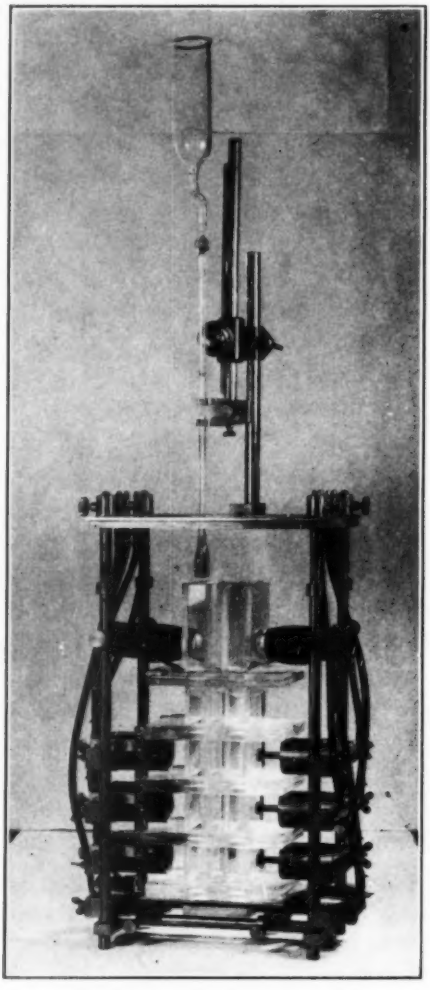


Fig. 22a—Large electrophoresis apparatus for preparative work.

the mobilities is the only decisive factor for the efficiency of the separation. It should be emphasized, however, that if the components combine partially in the solution, the separation will be incomplete also by this method. One should then look for a medium in which the compounds are likely to dissociate, for example extreme pH values.

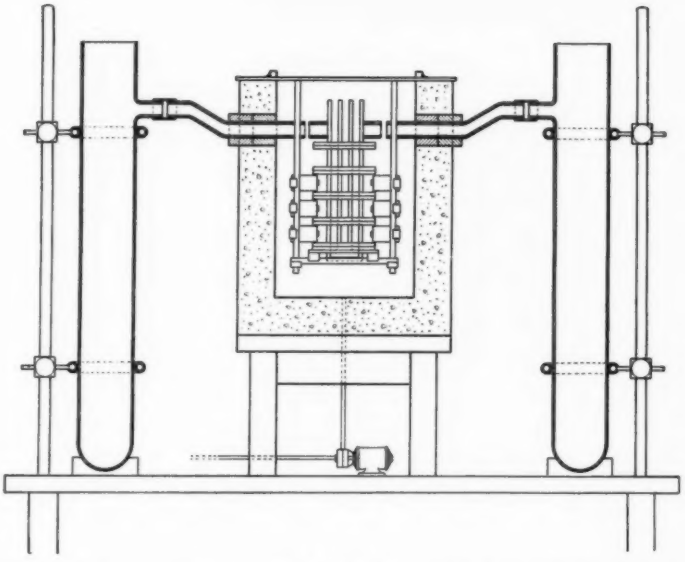


Fig. 22b—Large apparatus mounted in the bath, with the electrode vessels outside.

Even though successful separation may be obtained with the apparatus described, its capacity is too small for many purposes, the volume of the sample being only 10 cc. The value of electrophoretic methods for preparative work has been realized by many investigators, and several types of apparatus have been used, as has already been mentioned. For our purposes it has appeared essential to control the separation by optical observation, and an apparatus for this purpose taking 200-300 cc. is shown in Figure 22a. It has been built according to the same principle as the smaller type, with some modifications.[37]

The cross-sectional area is ten times as large, and the number of cells has been increased. Also the electrode vessel volume and the size of the electrodes must be increased accordingly. A special arrangement for sampling, making it possible to subdivide the contents of one compartment, has been introduced (Fig. 23). It consists of a pipette with a wide mouth, covered by a porous glass filter, which to a large extent eliminates convections when the liquid enters. The pipette is gradually lowered into one limb of the U-tube by the rack and pinion arrangement and its position with respect to the different boundaries is observed

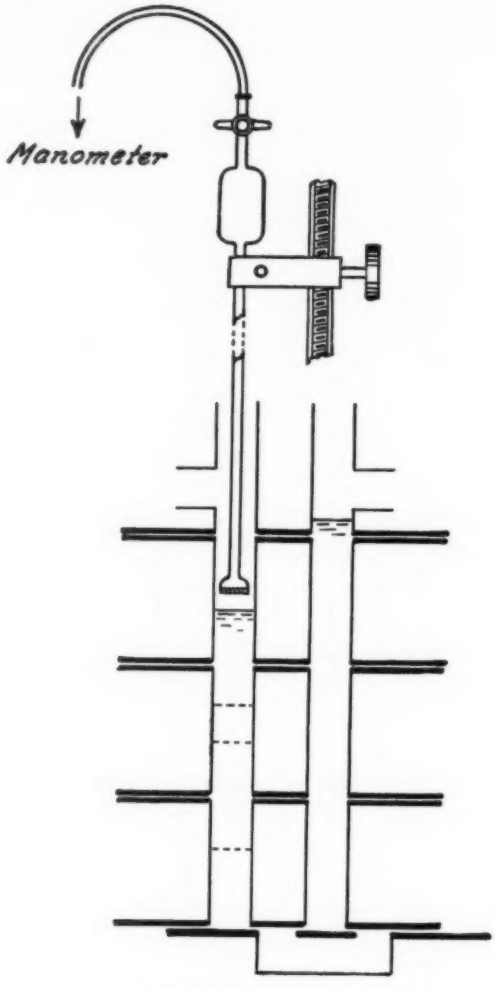


Fig. 23—Sampling device.

with the usual schlieren arrangement. Figure 22b shows the complete arrangement of the large apparatus. In preparative separation the so-called compensation arrangement has proved quite useful to increase the separation capacity of the electrophoresis tube (Fig. 24). In principle it depends upon the establishment of a slow and well-defined movement of the solution as a whole through the U-tube in a direction

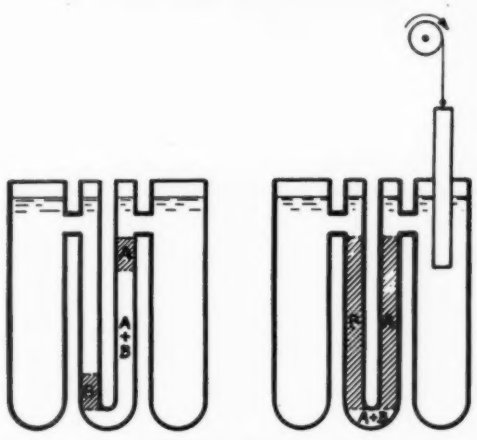


Fig. 24—Maximum separation obtainable without and with compensation.

opposite to that of the electrophoretic migration in order to keep the boundaries in the tube a sufficiently long time to attain as complete separation as possible.

The apparatus and procedure described have proved satisfactory for many separations, and have been used so far chiefly for serum fractionation. For many preparations where there are large differences in mobility and solutions of low conductivity can be used it seems probable that much simpler arrangements may work satisfactorily, especially if one is willing to sacrifice some of the convenience of this type of apparatus.

I may add that we also have a micro-cell (Fig. 25) with only a 2 cc. capacity, which has proved useful when only very small amounts of material are available.

In conclusion, I should like to say something about the limitations in the use of electrophoretic methods. It is well known that many large molecules, for example many polysaccharides, carry no charge and these, of course, fall entirely outside the scope of the method, except that their inability to move in an electric field at any pH is often quite useful to differentiate them as a group from other substances, as well as to separate them in quantity from other components in the mixture. To protein chemists it is a well-known fact that a very large number of albumins have isoelectric points in the neighborhood of pH 4.7. It has,

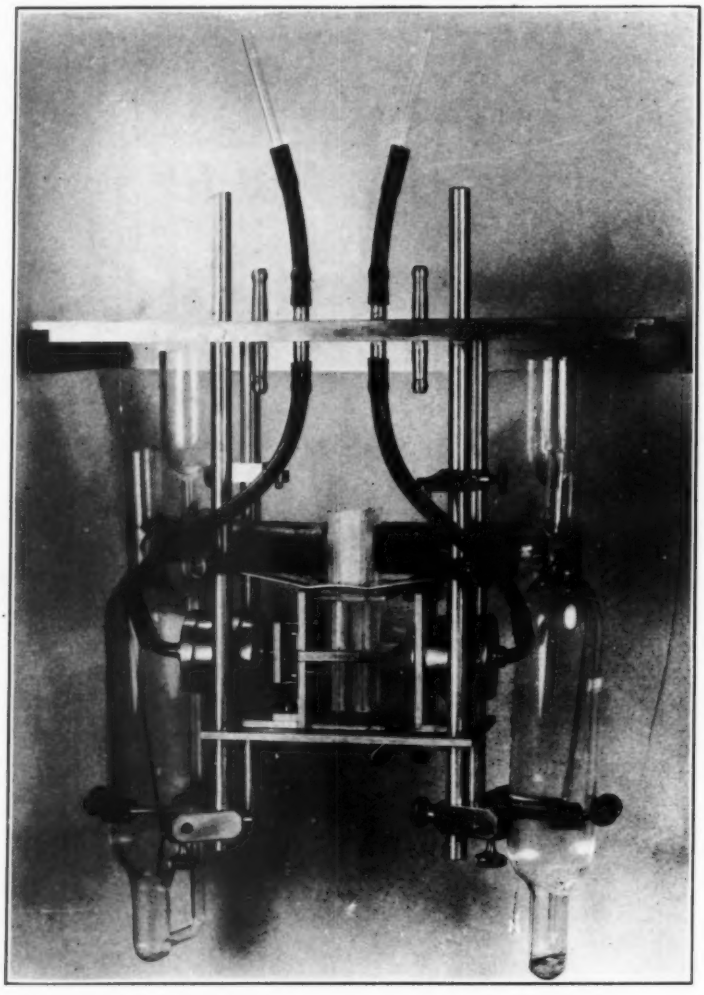


Fig. 25—Micro-electrophoresis apparatus (capacity 2 cc.).

however, been found that two proteins very seldom show the same mobility over the entire pH-stability range; usually the differences are most marked at an appreciable distance from the isoelectric point. Biologically related proteins are, of course, mostly quite similar, even if striking differences are encountered here too. The respiratory pigments

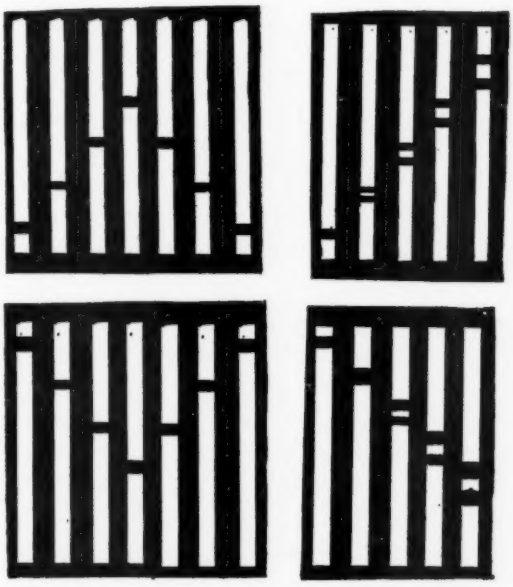


Fig. 26—Electrophoresis of guinea hen egg albumin (left) and a mixture of guinea hen and duck egg albumins (right). (From Landsteiner, Longsworth and van der Scheer[39]).

of the two snails, *Helix pomatia* and *Helix nemoralis* (hemocyanins) at pH 6.8 in 0.1 phosphate buffer show mobilities of 3.08 and 5.27 respectively (Pedersen;[38] Tiselius and Horsfall[23, 24]). Adair found that maternal and fetal hemoglobin from sheep could be distinguished easily (unpublished work). Landsteiner, Longsworth and van der Scheer[39] could differentiate egg albumins from the two groups of birds Anseriformes and Galliformes (Fig. 26). Within each group the species differences were, however, insufficient to give a distinct separation even if in some cases a broadening of the schlieren bands could be seen. As these authors emphasize, the species differences may often be located in groups which are electrically inert. There is hardly any doubt that serological reactions and solubilities of crystalline material are more specific properties than the electrophoretic mobility, even if the separation by these methods may not always be as clear-cut as that obtained by electrophoresis.

The greatest difficulty in preparative electrophoretic separation seems to be the spreading of the boundaries when they migrate over large distances. This spreading is much larger than can be accounted for by diffusion only, and, according to recent investigations by Horsfall and myself,[24] seems to be largely reversible, indicating either that most of the proteins investigated are still not quite homogenous or that an unknown source of disturbance is present in most of our experiments. If it were not for this effect, the possibilities of electrophoretic separation would be much greater, at least if sufficiently long times can be used for the experiments. It should be remembered that the separation is proportional to the time, whereas the diffusion is proportional only to the square root of time.

Another difficulty, which is of very great importance in quantitative work when mobilities have to be calculated or the relative amounts of the components estimated, is caused by the interference of the migration of the ions of the medium and is particularly important in solutions of high protein and low salt concentration. The differences between descending and ascending diagrams and the non-moving boundaries are caused by these effects. Time does not allow me to enter upon a discussion of these phenomena here. It may be sufficient, however, to emphasize the importance of an accurate knowledge of these effects. An excellent treatment of the subject has been given in a paper by Henry and Brittain[12] published some years ago.

Effects of this kind are also responsible for the difficulties encountered when it is tried to extend the use of electrophoretic methods to substances of low molecular weight like amino acids and the smaller polypeptides.

From what I have said I hope I have been able to demonstrate to you that the application of electrophoresis methods opens up some new possibilities of studying the constitution of native fluids under conditions which are at least somewhat closer to those prevailing in the native state than if the common, more drastic methods are applied. I believe that progress in this field will depend to a large extent upon the development of the experimental technique and upon the proper understanding of the significance of the results and the limitations of the method. This field should prove particularly fruitful for the collaboration of research workers in physical chemistry, biology, and medicine in a manner which has proved so valuable for many other problems of modern science.

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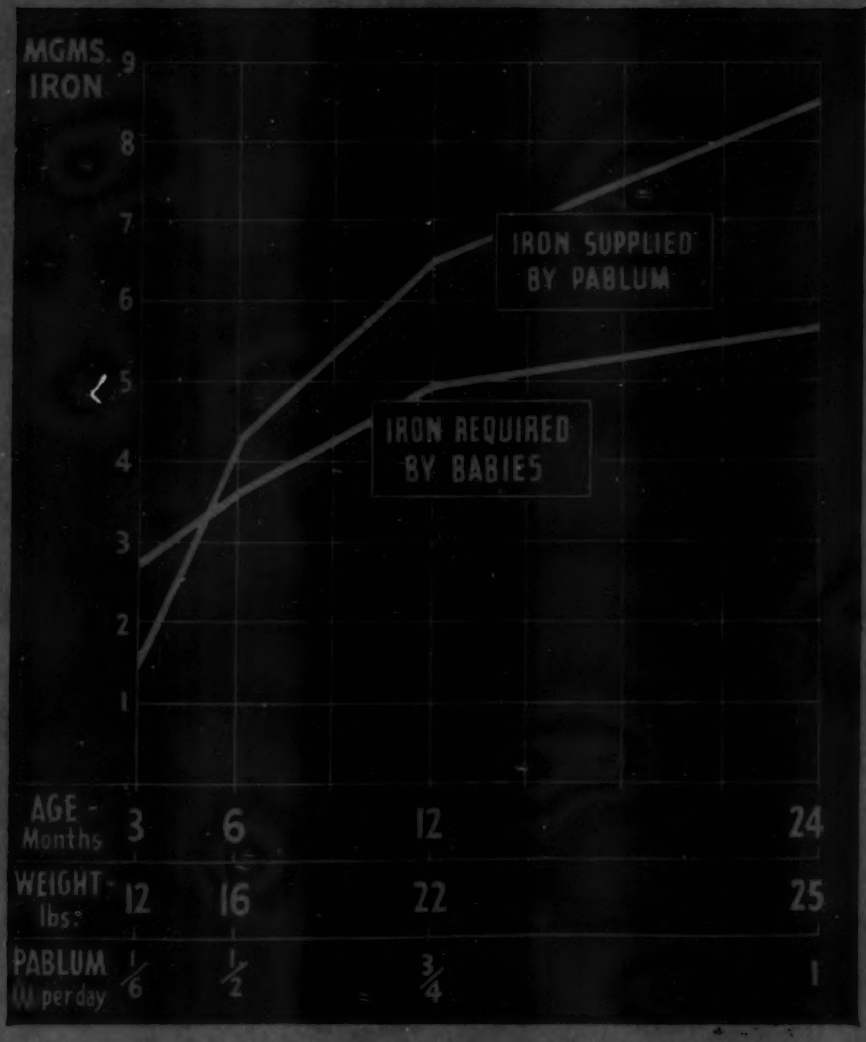
ARNE TISELIUS



DAILY AMOUNT OF IRON REQUIRED BY NORMAL BABIES, AND AMOUNT SUPPLIED BY PABLUM

The infant starts life with a store of iron. There is a steady drain of this reserve during the first few months. Because both breast milk and cow's milk are poor in iron, it is becoming the practice to feed iron-bearing foods at as early an age as possible. As shown in the above chart, from about the fourth month Pablum alone supplies more than the infant's daily iron requirements. In this chart, the requirements are based on the conservative estimate of the Council on Foods, i.e., 0.5 milligram per kilogram of body weight. The iron supplied by Pablum is calculated on the basis of 8.5 mg. per ounce. On account of its thorough cooking Pablum is well tolerated, having been fed without gastrointestinal upset as early as the first month. Bibliography on request. Mead Johnson & Co., Evansville, Ind., U.S.A.